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PETITION UNDER 37 CFR §1.182  
 Docket No. CIB-T105X  
 Serial No. 09/129,298  
 Group Art Unit 1638

 35,589  
 Jeff Lloyd, Patent Attorney

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit : 1638  
 Applicant(s) : Charles J. Arntzen, Peter B. Kipp, Ramesh Kumar, Gregory D. May  
 Serial No. : 09/129,298  
 Conf. No. : 4312  
 Filed : April 11, 2003  
 For : Use of Mixed Duplex Oligonucleotides to Effect Localized Genetic Changes in Plants

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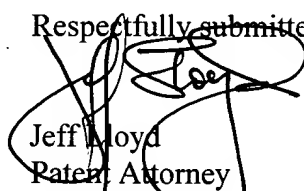
PETITION UNDER 37 CFR §1.182

Applicants have been requested to make a showing under 37 CFR §41.202(a)(4) that they are likely to prevail on priority for purposes of an interference being contemplated between the '298 Application and U.S. Patent No. 6,528,700 B1. Applicants believe that by the mere fact that their earliest provisional filing date of August 5, 1997 is more than three months earlier than the earliest possible priority date of the '700 Patent (which would be November 19, 1997), Applicants have made a sufficient showing that they are likely to prevail on priority, and should be designated as Senior Party in any interference between themselves and the Patentees of the '700 Patent. However, in an abundance of caution, and in case for some reason they are not accorded the benefit of their August 5, 1997 provisional filing date, Applicants have compiled Declarations and supporting exhibits which have been marked as Appendix A in their response

to the Official Communication of May 12, 2005 wherein Applicants were requested to make the required *prima facie* showing. The Appendix provides a sequence of events and dates which, if not necessary for their *prima facie* showing that they are likely to prevail on priority, would unfairly prejudice Applicants in the ensuing interference by forming a part of the record which the Junior Party '700 Patentees would have access to before any discovery phase of the ensuing interference had begun. Accordingly, if Applicants are to be accorded Senior Party status by being given the benefit of their August 5, 1997 provisional filing date, Applicants hereby petition for the return of Appendix A, such that it does not form a part of the official file history of the '298 Application. If this Petition is considered and granted, the appropriate fee as required by 37 CFR 1.17(f) is authorized to be deducted from Deposit Account No. 19-0065. The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicant invites the Examiner to call the undersigned if clarification is needed on any of the documents filed herewith, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



Jeff Lloyd

Patent Attorney

Registration No. 35,589

Phone No.: 352-375-8100

Fax No.: 352-372-5800

Address: P.O. Box 142950

Gainesville, FL 32614-2950

JL/amh

Attachments: Response Under 37 CFR 1.116; Petition and Fee for Extension of Time;  
Appendix A



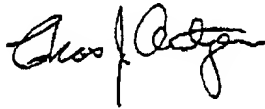
**Charles Arntzen**  
**7686 E. Wilderness Trail**  
**Superstition Mountain, AZ 85218-1806**  
**Phone : 480-288-1402**  
**Fax : 480-288-1406**  
**Email: charles.arntzen@asu.edu**

**Dr. Charles Arntzen Declaration**

I, Dr. Charles Arntzen, declare as follows:

1. I am a co-inventor of U.S. patent application serial number 09/129,298 filed on 5 August 1998 which claims the benefit of U.S. provisional patent application serial number 60/054,836 filed on 5 August 1997, hereinafter referred to as the "present application."
2. In 1996 I was the President of The Boyce Thompson Institute (BTI) at Cornell University, Ithaca, NY.
3. I assigned my rights to the above-identified patent application to Kimeragen, Inc.
4. Recently, I have been contacted by Dr. Keith Walker, CEO and Dr. Peter Beetham, Vice President of Research and Development, of Cibus LLC which is now responsible for the prosecution of the above-identified patent application. Drs. Walker and Beetham have summarized recent act prosecution activities including the possibility of a declaration of interference between the present application and US. Patent 6,528,700. I am familiar with the subject matter of the above-identified patent application.
5. In July 1996 at a meeting in Bar Harbor, ME, I met with a member of Kimeragen's Board of Directors and we discussed the chimeraplasty technology of Kimeragen and its possible application to plants. Within two weeks of that meeting I met with Dr. Gerry Messerschmidt and Dr. Ramesh Kumar of Kimeragen and we had more in depth discussions of the chimeraplasty technology. It was at this meeting where I suggested that single nucleotide mutations to the ALS gene, also known as the AHAS gene, will render the plant cell/plant resistant to sulfonylurea and/or imidazolinone herbicides. I also suggested chloroplast genome mutations that would result in resistance to triazine herbicides among other things. At this meeting we also discussed the possibility of doing a joint research collaboration between BTI and Kimeragen where the Kimeragen chimeraplasty technology would be studied in plants.
6. Over the next month or so BTI and Kimeragen exchanged drafts of a Research Collaboration Agreement. In October 1996 a research collaboration agreement was reached between Kimeragen and BTI. (001306 - 001309, 001318 and 001320 - 001326). Under this agreement, Kimeragen's primary responsibility was in the making of chimeras

(chimeric oligonucleotides) used to make mutations in the genome of a plant cell. BTI's primary responsibility was to deliver the chimeras into plant cells by precipitating the chimeras onto microparticles used in the gene gun delivery system to cause the mutation in a target gene within the genome of the plant cell. In some cases this involved regenerating a mutated plant. BTI immediately commenced work in October 1996 on mutating plant genes with mixed duplex oligonucleotides, even though Kimeragen did not send execution copies of the Research collaboration Agreement to BTI until 5 December 1996. (See 001343 - 001352) Dr. Greg May of BTI was the principal investigator and was also responsible for the administration of the research collaboration. Dr. May and graduate student Peter Kipp conducted much of the research in the Kimeragen program to successfully apply the technology to tobacco cells.



Dr. Charles Arntzen  
7686 E. Wilderness Trail  
Superstition Mountain, AZ 85218-7616

Date: November 11, 2005

**Patricia Avissar Declaration**

I, Patricia Avissar, declare as follows:

1. In October 1996 I commenced employment at Kimeragen, Inc. located in Newtown, PA as Research Associate.
2. I am aware of Kimeragen's U.S. patent application serial number 09/129,298 filed on 5 August 1998 which claims the benefit of U.S. provisional patent application serial number 60/054,836 filed on 5 August 1997, hereinafter referred to as the "present application."
3. Recently, I have been contacted by Dr. Keith Walker, CEO and Dr. Peter Beetham, Vice President of Research and Development, of Cibus LLC, which is now responsible for the prosecution of the above-identified patent application. Drs. Walker and Beetham have summarized recent prosecution activities including the possibility of a declaration of interference with US. Patent 6,528,700. I am familiar with the subject matter of the above identified patent application.
4. I reported to Dr. Ramesh Kumar, VP of Research and Development at Kimeragen. Dr. Kumar and I worked closely together during our employment at Kimeragen and in October 1996 Dr. Kumar and I had various conversations regarding Dr. Kumar's idea to use mixed duplex oligonucleotides to make mutations in the genome of a plant cell (chimeraplasty technology). These conversations included Dr. Kumar's idea for the use of the gene gun or biolistics to deliver the mixed duplex oligonucleotides into a plant cell in order to make the desired mutation. Those conversations also included discussions of mutating the ALS gene (also known as the AHAS gene) in order to develop herbicide resistant crops.

PA 11/10/2005

5. When I started employment at Kimeragen I was the first laboratory scientist hired and one of my main responsibilities was to oversee the start of the laboratory which had just been occupied by the company. These responsibilities included everything from setting up the laboratory facilities, ordering laboratory equipment and stocking the lab with miscellaneous supplies. I also was responsible for starting scientific projects and preparing work assignments for additional scientists to join Kimeragen. The next scientist hired by Kimeragen after me was Naomi Thomson.

6. My responsibilities in October 1996 and 1997 also involved my interaction with the team of scientists at BTI working on the Kimeragen collaboration. I would facilitate the ordering of oligonucleotides from Kimeragen's suppliers (and other collaborators) and forward them to BTI. I also conducted some sequencing experiments for BTI to confirm a mutation in the ALS gene as described in the following paragraph.

7. On 6 February 1997 I received PCR samples from Peter Kipp of BTI for sub-cloning and sequencing to confirm the mutations made in an ALS gene to make a mutant protein that conferred herbicide resistance to cells expressing the mutant protein. The full cloning and sequencing experiments and additional confirmation of sequence changes are reported on pages 144-165 of my Kimeragen laboratory notebook (See 002536-002557). The sequence alignments appear on pages 158-159 (See 002550 - 002552). These experiments confirm the mutation made to the ALS gene at the proline 196 position which resulted in a gene product that made the plant cell resistant to the sulfonylurea herbicide GLEAN.

8. At all times during my employment at Kimeragen the company and especially Dr. Kumar were diligent in pursuing the chimeraplasty technology in both the development and out-licensing of that technology.

Patricia Avissar

P. Avissar

11/10/2005

Cibus CONFIDENTIAL

DECLARATION

**Peter Kipp Declaration**

I, Dr. Peter Kipp, declare as follows:

1. I am a co-inventor of U.S. patent application serial number 09/129,298 filed on 5 August 1998 which claims the benefit of U.S. provisional patent application serial number 60/054,836 filed on 5 August 1997, hereinafter referred to as the "present application."
2. In 1996 I was a graduate student at Cornell University, Ithaca, NY in The Boyce Thompson Institute (BTI).
3. I assigned my rights to the above identified patent application to Kimeragen, Inc.
4. Recently, I have been contacted by Dr. Keith Walker, CEO and Dr. Peter Beetham, Vice President of Research and Development, of Cibus LLC which is now responsible for the prosecution of the above-identified patent application. Drs. Walker and Beetham have summarized recent act prosecution activities including the possibility of a declaration of interference between the present application and US Patent 6,528,700. I am familiar with the subject matter of the above identified patent application.
5. In October 1996 I accepted an assignment from co-inventor Dr. Charlie Arntzen, President of BTI to work under a Research Agreement BTI had with Kimeragen as part of my graduate studies. My work under this Research Agreement involved using mixed duplex oligonucleotides to make mutations in the genome of a plant cell herein after referred to as "chimeraplasty technology." In particular, I developed methodologies for delivering the oligonucleotides into plant cells with the gene gun or biolistics transformation and developed protocols for precipitating the oligonucleotides onto microparticles which were usually gold or tungsten. I also performed experiments to demonstrate that plant sequences could be mutated using

chimeraplasty technology and to determine the mechanism of action of the oligonucleotides in making the mutations in the plant genome.

6. My first experiments started on 22 October 1996 involved using a non-specific fluorescently tagged chimeric oligonucleotide to determine the precipitation characteristics of small molecular weight chimeric oligonucleotides on gold particles used in biolistics transformation and to determine the efficiency of biolistics introductions as well as cellular uptake using tobacco protoplasts. The gold precipitation using a standard protocol for the preparation of biolistic microparticles was unsuccessful. The cellular uptake protocols using an NT-1 (*Nicotiana tabacum* NT) cell culture were successful. (See 002188-002194)
7. On 7 November 1996 the first four chimeric oligonucleotides all designed to target the acetolactate synthase (ALS – also known as the acetoxyacid synthase or AHAS) gene were ordered by Dr. Ramesh Kumar of Kimeragen from Perkin Elmer. The chimeric oligonucleotides were denoted by ALS-1 (68MER), ALS-2 (68MER), AL-1DNA and ALS-3. (See 002613) The first two (ALS-2, ALS-1DNA) were received at Kimeragen on 9 Decemebr 1996. ALS-1 was received on the 10 December 1996 and ALS-3 was received on the 11 December 1996. (See 002610).
8. On 21 November 1996 I report the sequences of the polymerase chain reaction (PCR) primers designed to amplify the tobacco Acetolactate Synthase (ALS) gene. These were designed using sequence information from the Bedbrook publication in Embo Journal (7:1246) and they covered sequence from both the class I and II ALS genes as described in that paper. (See 002214 - 002215) The ALS PCR cloning was continued on 6 December 1996. (See 002222 – 002223).
9. On 22 November 1996 I conducted a precipitation test with the fluorescently tagged oligos and both tungsten and gold microparticles. The test was unsuccessful (See 002215 – 002216).
10. On 5 December 1996 I continued microparticle precipitation experiments using tungsten and tRNA (See 002219- 002222).

11. The first biolistics were performed on 9 December 1996 with the following samples: flowers (not sure where from), onion and NT-1 tobacco cells (See 002224). I reported this experiment as unsuccessful (See 002225).

12. On 10 December 1996 I set up a matrix of precipitation conditions to test oligonucleotide precipitation onto gold microprojectile particles. (See 002226 - 002229). These were reported to Kimeragen scientists (Dr. Ramesh Kumar et al) on December 12<sup>th</sup> with at least one being successful.

13. On 13 December 1996 I sequenced the ALS gene in tobacco from earlier clonings (See 002230 - 002232). This confirmed the two classes of ALS SurA and SurB alleles and the sequence around the targeted site -- Proline 196. I also initiated experiments to test the selectability of NT-1 tobacco cells on medium containing various levels of herbicide. The herbicide added was a sulfonyleurea known as "Glean". The kill curves are described on (See 002233 - 002234).

14. I then began the first introductions of targeting oligos (NB also known as RNA/DNA chimeric oligonucleotides, Chimeraplasts and Genoplasts). It is important to note that targeting oligonucleotides all based on the RNA/DNA design, are also known as "chimeras", "chimeraplasts" and "genoplasts". I prepared tobacco cells on 17 December 1996 and the biolistic precipitations were begun the next day. (See 002237 - 002238). The bombarded cells were allowed to recover in NT-1 medium. This experiment was performed using oligos, not identified by the collaborators at Kimeragen, it was a "blind" experiment. I had a vacation break from 19 December to 29 December 1996. In my absence co-inventor Dr. Greg May plated the bombarded cells on selection medium (containing 30 ppb Glean herbicide) on 20 December 1996. Upon my return from vacation on 30 December 1996 I report some growth on selection medium from various treatments. (See 002238, 002239).

15. I documented the calli growth 7 January 1997 (See 002244). Some of the calli were transferred to help eliminate escapes (See 002245). Next, I transferred the calli on 14 January 1997 (See 002255) where the calli were re-cultured on medium containing 50 ppb Glean. Some of the earlier calli on 30 ppb Glean were noted (January 15<sup>th</sup>) to be growing well, healthy and to "look different" (See 002257).

16. On 16 and 17 January 1997 I reported summaries of planned projects that I discussed with Dr. Greg May which included DNA/protein strand transfer assays, fluorescent labeling of chimeras using rhodamine and the progress for making a green fluorescent protein construct with a deletion or stop mutation. The delivery experiments discussed utilized NT-1 protoplasts. (See 002258 - 002269)

17. The first genomic DNA samples extracted from two samples of sulfonylurea tolerant calli were performed on 20 January 1997 (See 002270-002271). These samples were taken and labeled as ALS 2-3 and ALS 1-1. I observed that these two samples were regenerating on plates containing medium with 30 ppb Glean. (Note - NT-1 is known to be a non-regenerable cell line - so regeneration here would refer to calli growth).

18. On 20 January 1997 genomic DNA was used in a PCR reaction to generate amplified DNA primed from the tobacco ALS genes (See 002272 and 002273). The ALS 1-1 sample amplified well and the band formed on an agarose gel was removed and stored at 4°C. The ALS 2-3 sample had minimal amplification and was "re-amplified" (See 002274). Both samples were gel purified and quantified for direct PCR fragment sequencing (NB - controls were included) (See 002276 - 002277). The sequencing reactions and subsequent gel were reported on 22 January 1997 (pages 002277 - 002281). This was the first molecular characterization where there is an indication that the sequence at proline 196 has been modified utilizing a mixed duplex oligonucleotide. The sequence gel had an ambiguity at both cytosines in the CCA proline codon. This analysis is outlined on (See 002281).



19. On 23 January 1997 I had a conversation with Naomi Thomson (Kimeragen employee) where we discussed the ambiguity (this refers to the fact the gel is not clearly interpreted at a particular nucleotide or nucleotides) of the sequence at proline 196 and she suggested to re-run the sequencing reactions in the opposite direction. (See 002282) The next sequencing reactions were also performed on 23 January 1997 (See 002283). Unfortunately this sequence run was not as clear (See 002285). I then decided to have the ALS PCR products sequenced by Kimeragen. (See 002286)

20. On 24 January 1997 I recorded the sequences of the "chimeras" provided by Kimeragen for the blind experiments. At this stage the ALS-2 targeting the proline to leucine at position 196 seems to have produced the robustly growing calli denoted as ALS 2-3 now growing on 50 ppb. (See 002287) The additional calli needed to be further clarified with clear sequence so I worked on improving my PCR to ensure both tobacco ALS alleles were represented in the PCR amplified DNAs. (See 002291 - 002294).

21. I sent the improved PCR products to Kimeragen (Naomi Thomson) on 28 January 1997. (See 002295). I confirmed the sequences on 31 January 1997. (page 002300). The altered bases were confirmed to have changes at proline 196 codon for all samples (1-1, 1-2, and 2-3) sent to Kimeragen. The nucleotide alteration identified was shifted one nucleotide in the 5 prime direction.

22. I performed additional PCRs to confirm the 5 prime shift on 4 February 1997. (page 002306 and 002307) and again sent the PCR samples to Kimeragen for sequencing. The additional confirmation of changes was reported 5 February 1997. (See 002310). The final samples sent to Kimeragen for cloning and sequencing were noted on 5 February 1997. (See 002311).

23. The cloning described above confirmed the proline codon at position 196 in the ALS gene had been changed from CCA to either a TCA or an ACA codon that would change the proline to a serine or a threonine respectively. This was reported in the alignments on 18 February 1997 by Patricia Avissar of Kimeragen. (See 002552 - 002555). The combination of ALS sequencing by PCR and cloning had corroborated the genetic change in the chimera-plasty treated tobacco

samples at proline position 196. These tobacco samples also continued to grow on herbicide containing medium at BTI.

24. In February 1997 I continued with experiments introducing chimeraplasts into tobacco cells, tobacco leaves and calli (the leaves and calli were all the tobacco line know as "Sampson"). (See 002320 - 002321). These experiments were designed to discover different methods of chimeraplast introduction and regeneration of tobacco plantlets from these regenerable tissues.

25. I also worked on some Green Flourescent Protein (GFP) mutant strategies. The first descriptions of the mutant PCR primers used for this strategy were noted on 17 December 1996 after a discussion with Dr. Ramesh Kumar. (See 002236) The GFP work and tobacco ALS targeting continued after the first reduction of practice concurrently at BTI. The two mutants of GFP generated were a "stop" mutant (where a sequence was modified using plasmid site-specific mutagenesis to incorporate a stop codon in the GFP gene) and a "deletion" mutant where a frameshift mutation was incorporated at the same codon (codon 6 of the GFP coding sequence. The first mutants and sequence confirmation were reported on 13 February 1997. (See 002327 - 002328).

26. On 14 February 1997 I also reported additional resistant calli forming from the original tobacco experiment from 18 December 1996. I noted that these resistant calli formed much later than expected and noted that maybe the Glean herbicide had broken down. (See 002330). I continued to analyze surviving calli by PCR. These samples plus the original confirmed ALS "conversions" (name coined for successful chimeraplasty) were sent to Kimeragen on 4 March 1997. (See 002353).

27. The above described work was continued throughout 1997 and 1998 in collaboration with Drs. Greg May and Peter Beetham of BTI confirming the ALS conversions in NT-1 cells and GFP conversions in NT-1 cells.

Peter Kipp



Date:

11/10/05

**Ramesh Kumar Declaration**

I, Dr. Ramesh Kumar, declare as follows:

1. I am a co-inventor of U.S. patent application serial number 09/129,298 filed on 5 August 1998 which claims the benefit of U.S. provisional patent application serial number 60/054,836 filed on 5 August 1997, hereinafter referred to as the "present application."
2. In the Spring of 1996 I commenced employment at Kimeragen, Inc. located in Newtown, PA as Vice President of Research and Development.
3. I assigned my rights to the above identified patent application to Kimeragen, Inc.
4. Recently, I have been contacted by Dr. Keith Walker, CEO and Dr. Peter Beetham, Vice President of Research and Development, of Cibus LLC which is now responsible for the prosecution of the above-identified patent application. Drs. Walker and Beetham have summarized recent act prosecution activities including the possibility of a declaration of interference between the present application and US. Patent 6,528,700. I am familiar with the subject matter of the above identified patent application.
5. In May 1996 I conceived the idea of using mixed duplex oligonucleotides to make mutations in the genome of a plant cell herein after referred to as "chimeraplasty technology." My ideas included the use of the gene gun or biolistics as a means for delivering the mixed duplex oligonucleotides into plant cells and mutating plant genes to make a plant cell and plants regenerated therefrom herbicide resistant. These ideas are further described and embodied in the present application.

execution copies of the Research collaboration Agreement were not sent to BTI until 5 December 1996. (See 001343 - 001352) I worked closely with Dr. Greg May and Dr. Arntzen regarding the research the BTI team was doing for Kimeragen.

11. In February 1997, Naomi Thomson and Patricia Avissar, who both worked under my supervision at Kimeragen, confirmed mutations made in the ALS gene of tobacco cells. The mutated cells were made by BTI investigators working on the Kimeragen research agreement. The full cloning and sequencing experiments and additional confirmation of sequence changes are reported on pages 144-165 of my Kimeragen laboratory notebook (See 002536-002557). The sequence alignments appear on pages 158-159 (See 002550 - 002552). These experiments confirm the mutation made to the ALS gene at the proline 196 position which resulted in a gene product that made the plant cell resistant to the sulfonylurea herbicide GLEAN.

11. When I was hired by Kimeragen in the Spring of 1996, Kimeragen was a young start-up company that had no office or laboratory building. Soon after my employment began at Kimeragen I worked with Lisa Malseed who was a consultant to Kimeragen. Lisa's worked continuously from Spring 1996 in identifying building space for Kimeragen's office and laboratory at 300 Pheasant Run, Newtown, PA. Ms. Malseed negotiated a lease for that space for Kimeragen's office and laboratory and also negotiated with contractors to build out that space. In September/October 1996 Kimeragen moved into the offices and labs at 300 Pheasant Run, Newtown, PA.



Dr. Ramesh Kumar

Lisa Malseed Declaration

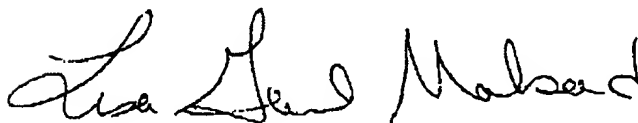
I, Lisa Gail Malseed, declare as follows:

1. On or about 1 July 1997 I commenced employment at Kimeragen, Inc. located in Newtown, PA as Senior Attorney. Prior to becoming a full-time employee I served as a consultant to Kimeragen from March 1996 to June 1997.
2. I am aware of Kimeragen's U.S. patent application serial number 09/129,298 filed on 5 August 1998 which claims the benefit of U.S. provisional patent application serial number 60/054,836 filed on 5 August 1997, hereinafter referred to as the "present application."
3. Recently, I have been contacted by Dr. Keith Walker, CEO and Dr. Peter Beetham, Vice President of Research and Development, of Cibus LLC, which is now responsible for the prosecution of the above-identified patent application. Drs. Walker and Beetham have summarized recent prosecution activities including the possibility of a declaration of interference with US. Patent 6,528,700.
4. Dr. Ramesh Kumar, VP of Research and Development at Kimeragen, Dr. Gerry Messerschmidt, President of Kimeragen, and I worked closely together during my employment at Kimeragen and during the time I served as a consultant. I reported to Jennifer Kmiec, Vice President of Administration.
5. Soon after I became a consultant to Kimeragen in March of 1996 I worked in obtaining a lease for an office and laboratory building for Kimeragen. I worked continuously through the summer of 1996 on this project and Kimeragen moved into its office and laboratory building at 300 Pheasant Run, Newtown, PA, in September/October 1996. Prior to the September/October 1996

opening of Kimeragen's office and laboratory the Kimeragen executive met at the home of Dr. Kumar or at locations in Princeton.

6. In addition to negotiating the lease for the new Kimeragen facility I also negotiated and drafted agreements with contractors to build out the office and laboratory space at Kimeragen's new facility.

7. At all times during my employment at Kimeragen and while I consulted with Kimeragen, the company and especially Dr. Kumar were diligent in pursuing the chimeraplasty technology in both the development and out-licensing of that technology in plants.

A handwritten signature in cursive script, reading "Lisa Gail Malseed".

Lisa Gail Malseed

November 11, 2005

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## DECLARATION

### Dr. Gregory D. May Declaration

I, Dr. Gregory May, declare as follows:

1. I am a co-inventor of U.S. patent application serial number 09/129,298 filed on 5 August 1998 which claims the benefit of U.S. provisional patent application serial number 60/054,836 filed on 5 August 1997, hereinafter referred to as the "present application."
2. In 1996 I was an Assistant Research Scientist at The Boyce Thompson Institute (BTI) at Cornell University, Ithaca, NY.
3. I assigned my rights to the above identified patent application to Kimeragen, Inc.
4. Recently, I have been contacted by Dr. Peter Beetham, Vice President of Research and Development, of Cibus LLC which is now responsible for the prosecution of the above-identified patent application. Dr. Beetham has summarized recent act prosecution activities including the possibility of a declaration of interference between the present application and US. Patent 6,528,700. I am familiar with the subject matter of the above identified patent application.
5. In October 1996 I accepted an assignment from co-inventor Dr. Charlie Arntzen, President of BTI, to manage a Research Agreement BTI had with Kimeragen. My work under this Research Agreement involved using mixed duplex oligonucleotides to make mutations in the genome of a plant cell herein after referred to as "chimeraplasty technology." I also supervised Peter Kipp, a graduate student at Cornell University, who did much of the actual laboratory work. In particular, Peter Kipp and I worked together to develop methodologies for delivering the oligonucleotides into plant cells with the gene gun or biolistics transformation. This included protocols for precipitating the oligonucleotides onto microparticles which were usually gold or tungsten. Peter Kipp and I also designed experiments to determine the mechanism of action of the oligonucleotides in making the mutations in the plant genome.



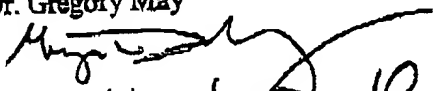
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**DECLARATION**

6. The work BTI did under the collaboration with Kimeragen commenced in mid-October when Kimeragen supplied the BTI lab with a 68 mer, fluorescently labeled chimeric oligonucleotide (AP-2M) under a Material Transfer Agreement (See 001143 - 001149)

7. On 20 December 1996 I plated tobacco callus cells on selection medium (containing 30 ppb Glean herbicide) for Peter Kipp who was on vacation. (002238)

Dr. Gregory May

  
Date: November 10, 2005

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Gerry Messerschmidt Declaration

I, Dr. Gerald Messerschmidt, declare as follows:

1. On or about 1 February 1996 I commenced employment at Kimeragen, Inc. as CEO and President.
2. Recently, I have been contacted by Dr. Keith Walker, CEO and Dr. Peter Beetham, Vice President of Research and Development, of Cibus LLC, which is now responsible for the prosecution of the above-identified patent application. Drs. Walker and Beetham have summarized recent prosecution activities including the possibility of a declaration of interference with US. Patent 6,528,700. I am familiar with the subject matter of the above identified patent application.
3. After I started work at Kimeragen the company conducted a job search for the position of Vice President Research and Development. That job search ended up with the hiring of Dr. Ramesh Kumar as VP R&D in the Spring of 1996.
4. Also, soon after starting employment at Kimeragen a top priority of mine was to locate and lease office and laboratory space for the company. I worked closely with Lisa Malseed in locating office space in Newtown, PA and negotiating terms of the lease agreement. Kimeragen moved into the leased space in September/October 1996.
5. Dr. Kumar and I worked closely together and in May 1996 Dr. Kumar and I had various conversations regarding Dr. Kumar's idea to use mixed duplex oligonucleotides to make mutations in the genome of a plant cell (chimeraplasty). These conversations included the use of the gene gun or biolistics to deliver the mixed duplex oligonucleotides into a plant cell and the

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modifications of endogenous plant genes to render the plant resistant to a herbicide. One such gene identified to be mutated to render a plant herbicide-resistant was the ALS gene which is also known as the AHAS gene.

6. Soon after Dr. Kumar started employment at Kimeragen one of his responsibilities was to develop chimera-plasty technology in plants and to out-license that technology to the agricultural industry.

7. On 31 May 1996 Dr. Kumar, Carroll "Bo" Allen, VP Business Development at Kimeragen, and I met with representatives of Pioneer Hi-Bred International in Johnston/Des Moines, IA to discuss the possibility of licensing Kimeragen's chimera-plasty technology to Pioneer for use in plants. Pioneer wanted to evaluate Kimeragen's technology in plants. (See 000002 - 000003) Present at that meeting on behalf of Pioneer were Peter Fuller, Dorothy Pierce, Chris Baszczyński, Ben Bowen and Bob Miclo. (See 000013-000016). Present for Kimeragen were myself, Dr. Kumar and Mr. Allen and the meeting was conducted under the terms of a confidentiality agreement. (See 000017-000018 and 000010-000012). On 6 August 1996 we had another meeting with Pioneer to discuss licensing Kimeragen's chimera-plasty technology to Pioneer. Present at that meeting were Dotty Pierce, Chris Baszczyński, Ben Bowen, Tony Cavalieri and Peter Fuller on behalf of Pioneer and myself, Dr. Eric Kmiec, Dr. Kumar and Mr. Allen on behalf of Kimeragen. (See 000023). By 31 August 1996 Stephen Johnson, Kimeragen's outside counsel had drafted a "draft" Pioneer License Agreement for review by Kimeragen executives. (See 000053-000068). On 10 September 1996 a draft agreement was faxed to Pioneer for their review. (See 000071) On 20 September Pioneer sent their comments on the License Agreement back to Kimeragen. (See 000087 - 000125) On 20 November 1996 Mr. Bo Allen sent a letter to Pioneer representatives indicating that a draft non-exclusive license agreement had not yet been prepared. (See 000324 - 000326) Then on 9 December 1996 Mr. Bo Allen and I met in Johnston/Des Moines with Tony Cavalieri and Peter Fuller of Pioneer to discuss a license agreement of Kimeragen's chimera-plasty technology. A final agreement was not reached with Pioneer in 1996 although the negotiations were continued into 1997.

Cibus CONFIDENTIAL

8. In 1997 Kimeragen and Pioneer continued negotiating a license to the Kimeragen chimeraplasty technology which resulted in a final signed license agreement on 10 March 1997. (See 000641 - 00067) On 12 March 1997 Pioneer and Kimeragen amended their license agreement twice with a First Amended and Restated License Agreement (000668 - 694) and a Second Amended and Restated License Agreement (000994 - 001021)

9. In late July 1996 Dr. Kumar, Mr. Allen and I met with Dr. Charlie Arntzen of The Boyce Thomson Institute (BTI) at Cornell University in Ithaca, NY. BTI is a world renowned plant biotechnology laboratory and Dr. Arntzen was at the time the President of BTI. The reason for our visit to BTI was to negotiate a research agreement/collaboration where BTI would work with Kimeragen scientists to perform research in the plant area using Kimeragen's chimeraplasty technology. In October 1996 a research collaboration agreement was reached between Kimeragen and BTI as drafts of a formal agreement were exchanged between the parties (001306 - 001309, 001318 and 001320 - 001326). BTI immediately commenced work in October 1996 on mutating plant genes with mixed duplex oligonucleotides while execution copies of the Research collaboration Agreement were not sent to BTI until 5 December 1996. (See 001343 - 001352)

10. At all times during my employment at Kimeragen the company and especially Dr. Kumar were diligent in pursuing the chimeraplasty technology in both the development and out-licensing of that technology in plants.



Dr. Gerald Messerschmidt

## **APPENDIX A**

## COVER SHEET TO APPENDIX A

To Response Dated November 14, 2005

In U.S. Serial No. 09/129,298; Filed April 11, 2003

This Appendix A including the accompanying Declarations and supporting documentation, is being submitted on a contingent basis, to be considered and form a part of the official record of the '298 Application **ONLY IF APPLICANTS ARE NOT DEEMED TO HAVE MADE A *PRIMA FACIE* SHOWING OF THEIR LIKELIHOOD TO PREVAIL ON PRIORITY BASED ON THEIR AUGUST 5, 1997 FILING DATE OF THEIR PROVISIONAL APPLICATION**, which was some three months earlier than the earliest priority date to which the '700 patent could possibly be entitled. If for some reason Applicants are **NOT** accorded the benefit of their August 5, 1997 priority date, **THEN AND ONLY THEN** are the following statements, accompanying Declarations, and supporting documents to be considered and form a part of the file history of the '298 Application. Assuming that the contents of this Appendix will **NOT** need to be considered at this time, Applicants submit herewith a contingent Petition Pursuant to 37 CFR 1.182 requesting that the entire contents of Appendix A be returned to Applicants and not form a part of this record.

Accompanying this statement are the Declarations of Charles J. Arntzen, Gerry Messerschmidt, Lisa Malseed, Patricia Avissar, Carroll Allen, Peter B. Kipp, Ramesh Kumar, and Gregory D. May. These Declarations establish the facts and circumstances of the creation of KIMERAGEN, the collaborations between the inventors on the '298 Application (Charles J. Arntzen, Peter B. Kipp, Ramesh Kumar, Gregory D. May), the collaborations between people at Kimeragen and the Boyce Thompson Institute at Cornell University, and the events leading to the ultimate actual reduction to practice of a species within the scope of the Count by Peter Kipp, and corroborated by Patricia Avissar of Kimeragen in February, 1997. The corroboration by Patricia Avissar of an actual reduction to practice of a successful method of using MDONs to introduce targeted mutations into a plant cell, causing specific mutations in the ALS gene which rendered the plant cell herbicide as of February, 1997, is 9 months earlier than the earliest priority date to which the '700 Patent is entitled. Accordingly, Applicants hereby show they are likely to prevail on priority.

EASYLINK 2129097L001 3MAY96 18:07/18:08 EST  
FROM: 478327 PIOSEED DMS  
333439 PIONEER HIBRED  
PIONEER DATA SYSTEMS (CRN: NONE)  
TO: 2015437670

From: BOWENB "BEN BOWEN, PIONEER HI-BRED: 515-270-3647/3367(FAX)"  
To: FAX#12015437670  
CC: FULLERB,BOWENB  
Subject: Jerry Messerschmidt at Kimeragen

FAX MESSAGE FOR: JERRY MESSERSCHMIDT, KIMERAGEN, INC.

FROM: BEN BOWEN, PIONEER HI-BRED INTERNATIONAL, INC.

Jerry:

Thanks for your call. I tried calling your mobile number, but I guess you were out of area already. Anyway, I hope this FAX gets to you on Monday, as you requested.

I am a Senior Scientist in the Gene Targeting Group here at Pioneer and our mission is twofold: 1) to direct transgene integration at specific sites within the genome of corn; and 2) to manipulate endogenous gene expression in corn by creating or exchanging alleles through transformation. Our preferred method for corn transformation involves microprojectile bombardment and we work closely with another technology development group working on transformation approaches that are not genotype-restricted, a factor that poses the second major bottleneck in our current product development plans.

Recently, Pioneer has developed a procedure for identifying plants with transposon insertion mutations in any chosen gene of interest. However, this system is complicated by messy genetic backgrounds and the difficulty in sorting out which mutant phenotypes actually cosegregate with the mutation of interest. We have an increasing need for rapidly generating and evaluating knockout mutations, because we have recently embarked on a 3-year collaboration with Human Genome Sciences from which we hope to identify a large proportion (50,000 or more) of all the expressed genes in corn.

From the foregoing, I am sure you can see why we are so interested in the technology that Eric Kmiec has been developing. We would like to invite both you and Eric to visit Pioneer sometime soon to discuss the potential of Pioneer evaluating this technology in plants, primarily corn. It would be good if Eric could give a technical overview in seminar-format and then we could spend time exploring ways in which it might be profitable for Pioneer and Kimeragen to interact. I would prefer to discuss this fairly informally and cover both technical and business perspectives. I expect you would like to find out more about Pioneer's business and how we interact with other companies through research collaboration, licensing, etc. To give you a better perspective, we can prepare a short presentation along these lines and take you for a tour through the research facility. Likewise, we would be interested in a Kimeragen business overview from either you or Eric.

If this sounds interesting, please let's try to fix a date for your visit. We should cover our meeting with a Confidentiality Agreement and Peter Fuller can take care of that once we hear back from you.

This coming Monday is very busy for me, but I will get my voice mail during the day. Unfortunately, I will be out of the office Tuesday-Thursday, so it

000002

may be Friday before we can touch base. Perhaps the best thing would be to schedule a call sometime on Friday afternoon (1-4pm, at your convenience). Otherwise, my FAX number is 515-270-3367 and my e-mail address is bowenb@phibred.com. Peter Fuller may also be able start the ball rolling on this prior to Friday.

Hoping to talk to you soon,

Regards,

Ben Bowen

000003



# KIRKLAND & ELLIS

PARTNERSHIPS INCLUDING PROFESSIONAL CORPORATIONS

Rosemary T. Langford  
To Call Writer Direct:  
212 446-4946

Citicorp Center  
153 East 53rd Street  
New York, New York 10022-4675

212 446-4800

Facsimile:  
212 446-4900

May 29, 1996

## VIA FACSIMILE

Peter Fuller, Ph.D.  
Pioneer Hybrid International  
7300 Northwest 62nd Avenue  
Johnston, Iowa 50134-1004

Re: Kimeragen Confidentiality Agreement

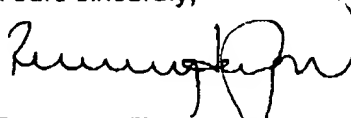
Dear Dr. Fuller:

Thank you for your message last Friday. As requested, I attach a form of mutual confidentiality agreement.

If the agreement is acceptable to Pioneer Hybrid International, please execute the enclosed two copies and fax me a copy of the executed version, with the originals to follow by courier for execution by Kimeragen. Alternatively, you may want to have the agreement executed for Kimeragen when representatives meet with you later in the week.

If you have any questions or issues relating to the attached form of agreement, please call me at (212) 446-4946.

Yours sincerely,



Rosemary T. Langford

RTL:cm  
Enclosures

cc: Gerald L. Messerschmidt  
Stephen Johnson

000010

Chicago

Denver

London

Los Angeles

Washington, D.C.

## CONFIDENTIALITY AGREEMENT

THIS AGREEMENT, is made on \_\_\_\_\_, 1996, by and between Kimeragen, Inc., a company incorporated in Delaware, having an address at 375 Park Avenue, Suite 1401, New York, New York 10152, and Pioneer Hybrid International, having an address at 7300 Northwest 62nd Avenue, Johnston, Iowa 50134-1004 ("PHI").

WHEREAS, the parties each have certain confidential technical and business information which they have disclosed and/or desire to disclose to one another on the terms and conditions set out in this Agreement.

### THE PARTIES AGREE:

1. **CONFIDENTIALITY** Each party hereby agrees (i) not to use any the information that is disclosed to it by, or received by it from, the other party (the "Confidential Information") except for the purpose of internal company discussions with a view to a possible agreement between the parties hereto (the "Purpose"); (ii) not to disclose the Confidential Information or any part of it to any person other than as permitted in this Agreement; and (iii) not to copy or duplicate the Confidential Information or any part of it except to the extent necessary for the Purpose.

2. **EXCEPTIONS** Clause 1 does not apply to any part of the Confidential Information disclosed by one party to the other (a) which is agreed in writing by the disclosing party to be excluded; or (b) which the receiving party can show was known to or developed by it prior to disclosure to receiving party by disclosing party; or (c) which is public knowledge, or becomes public knowledge in the future, other than through acts or omissions of the receiving party in breach of this Agreement; or (d) which is lawfully obtained by receiving party from sources independent of the disclosing party who have a lawful right to possess and disclose such Confidential Information; or (e) which it is necessary for the receiving party to disclose in order to comply with any applicable law or if required to do so by order of any court or any other judicial or administrative body, provided that prior to making such disclosure the receiving party gives the disclosing party notice of the requirement of disclosure and the information to be disclosed.

3. **EMPLOYEES** The receiving party in each case may disclose Confidential Information to such of its employees and consultants as necessary to carry out the Purpose and who are bound to that receiving party under confidentiality obligations.

4. **NO LICENSE** Each party retains all rights in its Confidential Information and the parties agree that their respective disclosures of Confidential Information hereunder shall not constitute any grant, option, or license to the other party of the Confidential Information or of any other intellectual property rights (including, without limitation, patents, registered and unregistered copyright, registered and unregistered trademarks, trade names, and mask works), now or hereinafter held by the disclosing party in each case.

5. **RETURN** Upon completion of the evaluation contemplated by the Purpose, or upon earlier request by KIMERAGEN, PHI shall (1) cease to use all Confidential Information provided or disclosed to it by KIMERAGEN ("Kimeragen Confidential Information"); (2) deliver to KIMERAGEN or destroy all Kimeragen Confidential Information contained in any tangible form held by PHI, and where such Kimeragen Confidential Information is destroyed, provide to KIMERAGEN a notice certifying destruction; and (3) delete permanently all Kimeragen Confidential Information in any electronic form held by PHI, provided that this clause does not apply to Kimeragen Confidential Information which, at the time of the request made by KIMERAGEN, came within the scope of Clause 2.

6. **AMENDMENTS** No amendment or variation to this Agreement is valid or binding on a party unless made in writing and executed by the parties.

7. **GOVERNING LAW** This Agreement shall be governed by, and construed and interpreted in accordance with, the laws of the State of New York, United States of America, without reference to conflict of laws principles.

8. **ENTIRE AGREEMENT** This Agreement constitutes the entire and exclusive agreement between the parties with respect to the subject matter hereof and supersedes and cancels all previous registrations, agreements, commitments and writings in respect thereof.

9. **AUTHORITY** The undersigned are duly authorized to execute this Agreement on behalf of KIMERAGEN and PHI as applicable.

IN WITNESS WHEREOF each party has executed this Agreement:

KIMERAGEN, INC.

PIONEER HYBRID INTERNATIONAL

By: \_\_\_\_\_

Name:

Title:

Date:

By: \_\_\_\_\_

Name:

Title:

Date:

5-31-96

Pioneer Hi-bred International

Peter Raller

Dorothy Pierce

Chris

Ben Bowen

Bob Miele

Peter Raller - overview of Company

Seeds

Services to Customer

Corn, Soybean, Sunflower, Canola  
haveotech efforts

Alfalfa, Sorghum, wheat  
no biotech effort yet,  
will access from  
outside

Technology Goals

Transformation  
efficiency  
Germ Plasm

FAST

Corn Genome

Molecular Marker

Gene Targeting/Recombination

Gene Expression

ANALYTICAL & diagnostic tool

000013

1. ID Candidate Genes  
Gene Discovery  
Treat Utility System
2. Manipulate Gene & Pathways  
Gene Targeting  
Other Molecular Technologies
3. ↓ # of Transforms  
Gene Targeting  
Other Technologies
4. Direct Integration  
Gene Targeting
5. Deliver candidate genes to germplasm  
Efficient Transformation
6. Make Sterility  
Male Sterility  
Gene Targeting
7. Molecular Targeting  
ASAT  
Gene Targeting

## Gene Targeting

- ↓ variability of transgene expression
- insert transgene to desired site
- ability to stack genetically engineered traits
- non-pulsed endogenous genes
- link value added traits with quantitative trait loci (QTL's)
- Allelic replacement
- Alternative to trait utility system for corn for studying function of known genes.

## Possible Markers

- turn on/off pigment system
- GFP

Stephan Johnson - "Wild Gun"  
"Open Heart Surgery"

TRACT Utility

Metabolic Gene

Enzyme Biochem

**KIMERAGEN, Inc.**

375 Park Avenue, Suite 1401

New York, N.Y. 10152

Phone: (212) 319-3216

Fax: (212) 319-2808

Direct Phone: (201) 635-1563

Direct Fax: (201) 635-1643

**Carroll "Bo" Allen****Senior Vice President****Business Development and Marketing**

June 3, 1996

Peter A. Fuller, Ph. D.

Director,

Technology Discovery and Assessment

Pioneer Hi-Bred International

7300 NW 62nd Ave., P.O.Box 1004

Johnston, Iowa 50131-1004

Dear Peter,

On behalf of Dr. Messerschmidt, Dr. Kumar, and myself, I would again like to thank you for your wonderful hospitality. The presentations by Chris, Ben, Dotty, and Robert were very interesting and insightful. We came away from our meeting very excited about the prospects for a collaboration. Again it is worth noting that all of our conversations are covered under the aegis of confidentiality. As I mentioned to you on Friday, I thought it might be useful to affirm Kimeragen's understanding of our next steps towards a formal collaboration.

First, you will caucus with your team to review three potential areas of opportunity for a mutual collaboration:

- using the technology to efficiently change traits in plants
- utilization of the technology as a research tool
- exploiting the technology to exchange "*bigger pieces of DNA*" in the genome

Based on the results of these meetings, Pioneer will determine the merits of continuing with discussions towards entering into a research and development agreement with Kimeragen.

Kimeragen has four basic tenets that we would like to negotiate into an agreement with Pioneer. We would like:

- a long term research agreement ( approximately 10 years )
- a licensing fee ( cash and / or equity ) for access and utilization of the technology

000017



Fuller Letter  
page2

- a prescribed royalty from technology enhanced , marketed products and / or technologies
- exclusive rights to all developed technologies that are applicable to human and animal utilization

Under this agreement Kimeragen will design and manufacture chimeras targeted to Pioneer's selected DNA sequences. This will be done in as timely and efficient manner as possible. Kimeragen will collaborate fully with Pioneer in all chimera based research ventures such as expanding the size of the DNA sequences being replaced.

Our expectations from a potential partner include aggressively researching the technology in multiple plant species, developing profitable applications for the technology, and registration and aggressive marketing of the products derived from the technology. Based on the people we met and our interchange of information and ideas, we believe that Pioneer is the kind of partner we are seeking for a collaboration in agronomy. We look forward to additional discussions moving us towards an agreement. Please feel free to contact me at any time ( 201-635-1563) should questions arise on business issues and Dr. Kumar for scientific issues (609-737-7319).

Regards,

Carroll "Bo" Allen  
Senior Vice President

000018

# KIMERAGEN & PIONEER MEETING AGENDA

August 6, 1996

#Reid-ConfA1 & #Reid-ConfA2

*genomics group*  
*Dotty Chris Dan*  
 "Technical Group": Pierce, Baszczynski, Bowen, Kmiec & Kumar  
*Genomics Meeting*

"Business Group": Cavaliere, Fuller, Messerschmidt & Allen

8:00	Greetings, introductions and purpose of the meeting (juice, coffee and bagels)	Bowen & Fuller #Reid-ConfA1
8:15	Informal seminar and general technology discussion	Dr. Kmiec, Technical & Business Groups, Technical guests #Reid-ConfA1
9:45	Break	All #Reid-ConfA1
10:00	Introductory comments by Tony Cavaliere & discussion about the goals of the Technology Group Meeting	Technical & Business Group #Reid-ConfA1
10:15	Identification of research goals and development of research plan	Technical & Business Group #Reid-ConfA1
12:00	Lunch (Dr. Kmiec to Airport)	Technical & Business Group #Reid-ConfA1
12:45	Kimeragen and Pioneer groups meet separately	Technical Group #Reid-ConfA1 & A2
1:15	Business Discussions & Conference call with Kimeragen legal counsel	Business Group & Yates #Reid-ConfA2
1:15	Technical Discussion (contd.) & Identification of research goals and development of research plan	Technical Group #Reid-ConfA1
4:30	Reconvene Technical Group and Business Group, discuss next steps	Technical & Business Group #Reid-ConfA2
5:00	Adjourn	

000023

Draft 8/31/96

## LICENSE AGREEMENT

THIS LICENSE AGREEMENT (the "Agreement") is made and entered into this \_\_\_\_\_ day of \_\_\_\_\_, 1996 (the "Effective Date") between Pioneer Hi-Bred International, Inc., an Iowa corporation of 700 Capitol Square, 400 Locust Street, Des Moines, IA 50309-2340 ("Licensee"), and Kimeragen, Inc., a Delaware corporation, of 300 Pheasant Run, Newtown, PA 18940 ("Licensor").

WHEREAS, Licensor is the exclusive licensee of Thomas Jefferson University ("TJU") under U.S. and foreign patent applications with respect to a chimeric vector for application in gene therapy developed by TJU, certain methods and processes using that chimeric vector, and the products of further research conducted at TJU and certain other institutions with respect to that chimeric vector; and

WHEREAS, Licensee wishes to obtain an exclusive, worldwide license under such technology to use and sell certain Licensed Products (as defined below).

NOW THEREFORE, in consideration of the mutual promises and covenants set forth herein and for good and valuable consideration, the adequacy and sufficiency of which is hereby acknowledged, the parties hereby agree as follows:

### ARTICLE 1--DEFINITIONS

1.1 "Affiliate" shall mean any company or entity related to Licensee by contract and through which Licensee produces and/or markets seed to end-user customers, but only for the period or periods during which such a contractual relationship exists.

1.2 "Chimera" shall mean the chimeric vector of RNA and DNA as claimed in the Patent Rights as supplemented by non-patented Licensor Technology.

1.3 "Confidential Information" shall mean all information, in whatever form, which is disclosed by either party prior to or subsequent to the Effective Date of this Agreement and which relates in any way to the Licensor Technology. Confidential Information shall not include information that the receiving party can demonstrate by written evidence: (a) is in the public domain (provided that information in the public domain has not or does not come into the public domain as the result of disclosure by the receiving party); (b) becomes available to the receiving party on a non-confidential basis from a source other than the disclosing party; or (c) is known to the receiving party prior to disclosure by the disclosing party.

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1 1.4 "Intellectual Property" shall mean without limitation, all filed patents,  
3 patentable inventions, know-how, trade secrets, techniques and ideas and all technical  
5 material which further includes germplasm, cell lines, plants, seeds, DNA and RNA  
sequences and genes, oligos and proteins, and associated methods and all applications  
therefor.

7 1.5 "Licensed Products" shall mean products containing genetic material that has  
9 been altered by the use of the Licensor Technology.

11 1.6 "Licensee Field-of-Use" shall mean all uses, excluding Pharmaceutical Use,  
13 in Corn, Sunflower, Soybean, Canola, Alfalfa and Sorghum and Research Use in Tobacco  
and [Arabidopsis?], provided that no Tobacco or [Arabidopsis] related in any way to  
such Research Use shall be sold by Licensee.

15 1.7 "Licensee Technology" shall mean all Intellectual Property conceived,  
17 controlled by, and/or developed by Licensee pertaining to the Chimera and associated  
methods required for its use.

19 1.8 "Licensor Field-of-Use" shall mean all uses outside the Licensee Field-of-Use.

21 1.9 "Licensor Technology" shall mean all Intellectual Property conceived,  
23 controlled by, and/or developed by Licensor pertaining to the Chimera and associated  
methods required for its use.

25 1.10 "Patent Rights" shall mean patents and patent applications and all foreign  
27 counterparts thereof in the Territory as listed in Exhibit A, including all future divisionals and  
29 continuations, continuations in part, additions, confirmations, renewals, extensions and  
reissues of patents and patent applications and their equivalents related to the Chimera  
and Licensor Technology.

31 1.11 "Plants" shall mean multicellular rooted organisms containing chlorophyll and  
33 cellulose cell walls.

35 1.12 "Pharmaceutical Use" shall mean all uses related to pharmaceutical  
37 compounds that are or would be regulated by the FDA Centers of Biological Evaluation and  
Research (CBER), Drug Evaluation and Research (CDER), Devices and Radiologic Health  
(CDRH) and Veterinary Medicine (CVM) in any species.

39 1.13 "Product Use" shall mean use of the Licensor Technology where the actual  
41 product sold does contain genetic material that has been altered by the use of the Licensor  
Technology.

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[8/31/98 6:04 pm]

1.14 "Research Use" shall mean use of the Licensors Technology where the actual product sold does not contain genetic material that has been altered by the use of the Licensors Technology.

1.15 "Sublicensee" shall mean any company or entity which is sublicensed by Licensee pursuant to the terms of Section 2.2.

1.16 "Subsidiary" shall mean any company or entity ~~at least 50% of~~ whose voting stock is owned or directly or indirectly controlled by Licensee. *OK?*  
*OK*

1.17 "Territory" shall mean the world.

## ARTICLE 2-LICENSE

2.1 Grant of License. Licensors hereby grants to Licensee, upon the terms and conditions set forth herein, a royalty-bearing, exclusive license under the Licensors Technology including the Patent Rights to (a) use and have used Chimera for Research Use, and (b) use and have used Chimera for Product Use, in both cases to the extent necessary to commercialize Licensed Products in the Licensee Field in the Territory. Licensee shall have the right to subcontract to Licensee's Subsidiaries and Affiliates activities relating to the growing and marketing of Licensed Products for the purposes of conducting Licensee's ongoing business. Such subcontracting shall not be considered a sublicense, provided that Licensee shall be responsible for the activities of such Subsidiaries and Affiliates as if they were part of Licensee.

2.2 Sublicensing. Licensee may grant sublicenses within the scope of the licenses granted in Section 2.1 to third parties, provided that:

- (a) Licensee shall cause all such sublicensees to comply with the provisions of this Agreement in the same manner as Licensee is bound hereby; *Sub licensee does not have right to sublicense?*
- (b) Licensee shall be responsible for compliance by such sublicensees with the terms of this Agreement; and
- (c) Licensee shall give the Licensors prompt written notice of all sublicenses granted pursuant to this Section 2.2.

2.3 Improvements. Licensee hereby grants to Licensors an exclusive paid up worldwide license to the Licensee Technology, with the right to grant sublicenses (which may in turn include the right to grant further sublicenses), for all uses and applications in the Licensors Field-of-Use, provide that Licensors shall have no right to Licensee Technology

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[8/31/98 6:04 pm]

1 for Plants. Licensor shall license Licensee to use improvements in Licensor Technology  
2 pursuant to Section 2.1. During the term provided in Section 6.2, the parties shall promptly  
3 disclose to each other any inventions or improvements which relate to the Licensor or  
4 Licensee Technology.

5  
6 2.4 Limitations on Exclusivity. Except as provided below, the licenses of  
7 Technology granted under Sections 2.1 and 2.2 shall be exclusive in the Territory in the  
8 respective field of use of each party.

9  
10 (a) The license shall be non-exclusive to the extent that rights of either party in  
11 the future shall be non-exclusive;

12 (b) The licenses shall be non-exclusive to the extent required by government  
13 regulations; and

14 (c) The licenses shall be non-exclusive to the extent of Research Use[?]. *in laboratories as defined in ABI AGREEMENT*  
15  
16

17  
18 2.5 Freedom to Use. In the event Licensor shall license third-party Intellectual  
19 Property to gain freedom-to-operate in Licensor's Field-of-Use and such Intellectual  
20 Property is also needed by Licensee, Licensor shall use all reasonable efforts to license  
21 such Intellectual Property so that Licensee's use of Licensor Intellectual Property is  
22 covered.

23 *Does not have right to make*  
24 2.6 Protection of Technology. Licensee shall not use the Licensor Technology  
25 for any purpose other than to make, use and sell Licensed Products, as provided in this  
26 Agreement. Licensee shall take no action in respect of the Licensor Technology which is  
27 inconsistent with the terms of the license granted under this Agreement.

28  
29 2.7 Acknowledgment of Rights. Licensee acknowledges that Licensee's right to  
30 use the Licensor Technology arises only out of the sublicense granted under this  
31 Agreement. All Licensed Products manufactured under issued patents shall bear a patent  
32 notice on the label as may be necessary or appropriate under the laws of the Territory as  
33 reasonably specified by Licensor to Licensee.

34  
35 2.8 Reservation of Rights. This Agreement contains no grants under any  
36 Intellectual Property of Licensor, except to the extent of those explicitly granted under  
37 Section 2.1.

38  
39 **ARTICLE 3—PAYMENT**

40  
41 3.1 Payment. In consideration of the rights granted herein, the Licensee shall pay  
42 to the Licensor.  
43

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[8/31/98 8:04 pm]

by wire transfer on dty

- 1 (a) three million dollars (US\$3,000,000) ~~within fifteen (15) days~~ of  
3 execution of this Agreement, provided that this Agreement shall not  
5 become effective until such payment shall be made.  
7 (b) two million dollars (US\$2,000,000) twenty (20) months after the  
9 Effective Date provided that Licensee shall not have issued a notice  
11 of termination pursuant to Section 6.3.  
13 (c) a royalty per bag of Licensed Product as specified on Exhibit B.  
15 (d) fifty percent (50%) of the value of all sublicense revenues (including  
17 fees and royalties) as provided in Section 3.3.

3.2 Royalty Conditions. Royalties shall:

- 17 (a) be payable only on the sales of Licensed Product in countries where  
19 pending or issued patents [or plant variety protection] exists  
21 covering the Chimera [or Licensed Products] or on sales of  
23 Licensed Product where the Licensed Product was developed or  
25 grown in countries where such rights exist;  
27 (b) accrue upon the sale of Licensed Product as recognized by the SEC  
29 [please explain];  
31 (c) be due and payable within sixty days after the close of Licensee's  
33 fiscal year (August 31);  
35 (d) not be due and payable on Licensed Products used for Licensee's  
37 internal purposes (test beds) but shall be due on samples provided to  
39 customers;  
41 (e) be prorated based on the package sizes shown on Exhibit B for  
43 package sizes not shown on Exhibit B.

3.3 Sublicenses. Licensor shall be entitled to share in all sublicensing fees and royalties on a 50:50 basis. In the event that Licensee barter or trades a Sublicense of Licensor Technology for other technology, or otherwise shall receive non-cash consideration, no fees shall be due to Licensor to the extent of such non-cash consideration but Licensee shall be obligated to pay royalties to Licensee on sales by the Sublicensee as if such sales were sales by Licensee. Licensee shall be responsible for monitoring and reporting on the sales of its Sublicensees and shall be responsible for all royalties due to Licensor.

↑ Didn't we agree on a higher royalty?  
Let's put in 75%

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**3.4 Reports.** Licensee shall provide royalty reports to Licensor within sixty (60) days after the close of Licensee's fiscal year, such reports shall contain information about Licensee's sales as well as sales by Sublicensees, including:

- (a) A listing of all Licensed Products sold by Licensee (including Subsidiaries and Affiliates) and Sublicensees, sorted by individual product, crops species and country;
- (b) Royalty calculation; and
- (c) Royalties due and payable.

**3.5 Audit.** The Licensee shall keep full and accurate records at its principal place of business in the United States of all Licensed Products grown, distributed, or sold by Licensee and its Sublicensees during the term of this Agreement. Such records shall be open, at reasonable times, for three (3) years following the end of the fiscal year to which they pertain, to an inspection by an independent certified public accountant acceptable to Licensee and retained by Licensor, at Licensor's expense, for the purposes of verifying Licensee's royalty payments under this Agreement. If a discrepancy of greater than five percent (5%) is found in any payment due hereunder, Licensee shall reimburse Licensor for the cost of such inspection and promptly pay such overdue amount **[together with interest at the rate specified in Section 3.7]**. The independent certified accountant shall report to Licensor only those items necessary to verify the royalty payments.

3.6 Third Party Royalties. In any case where use of the Licensor Technology licensed to Licensee is in future subject to a royalty to a third party other than TJU or another current licensor of Licensor (whether lump-sum or payable by reference to sales), then in the event that Licensee determines to use such Licensor Technology, Licensee shall in addition to the royalty specified in Section 3.1 be responsible for payment to Licensor of a further amount equal to the royalty payable to the third party attributable to sales by Licensee.

**[3.7 Late Payments.** All payments to be made by the Licensee to the Licensor hereunder shall bear interest at the prime or equivalent rate as quoted by Citibank N.A., New York, New York, on the day the payment is overdue plus two percent (2%) per annum until paid.]

## ARTICLE 4--PATENTS

4.1 Prosecution. Licensor shall use reasonable efforts to prosecute the Patent Rights to the extent within the control of Licensor. In the event that Licensor shall elect not to continue prosecution such that the Chimera would not be subject to patent protection,

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*I need to understand!*

1 then Licensee shall have the right to assume such prosecution at its expense, but any  
3 royalty obligation relating thereto shall cease.

5 4.2 Infringement. Licensee shall notify Licensor promptly of any action, claim or  
7 threat of patent infringement suit, either oral or written, or the commencement of any such  
9 patent infringement suit against Licensee relating to the Licensor Technology or of any  
infringement of the Patent Rights within the Licensee Field. The parties shall cooperate in  
the development and execution of a strategy to defend against such any action against  
Licensee or to prevent any such infringement by a third party.

## 11 ARTICLE 5--WARRANTIES & INDEMNITIES

### 13 5.1 Warranties.

15 (a) Each party represents and warrants to the other that it has full right,  
17 power and authority to enter into this Agreement and that the terms of  
19 this Agreement do not conflict with any other contractual obligations  
it has.

21 (b) Licensor represents and warrants that, as of the execution date, it  
23 knows of no Intellectual Property that would prevent Licensee from  
practicing the Licensor Technology.

25 (c) Licensor represents and warrants that it has the freedom to enter into  
27 this Agreement and the right to provide the rights and license  
contemplated in Section 2.1.

29 (d) No Other Warranties. Licensor makes no other warranty or  
31 representation with respect to the Licensor Technology, nor is  
33 Licensor in any way responsible for the utility of any Licensor  
Technology. LICENSOR HEREBY EXPRESSLY DISCLAIMS ANY  
35 AND ALL WARRANTIES AND REPRESENTATIONS, EXPRESS OR  
IMPLIED, ARISING BY LAW OR CUSTOM, WITH RESPECT TO THE  
37 LICENSOR TECHNOLOGY, INCLUDING, WITHOUT LIMITATION,  
WARRANTIES OF MERCHANTABILITY, FITNESS FOR A  
39 PARTICULAR PURPOSE OR NON-INFRINGEMENT. LICENSOR  
DOES NOT IN ANY WAY PROMISE THAT THE LICENSOR  
TECHNOLOGY SHALL PRODUCE ANY PARTICULAR RESULTS,  
PRODUCTS OR PROFITABILITY.

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1  
3  
5.2 Indemnities.

- 5 (a) Licensee hereby waives any claim against Licensor and TJU and  
7 agrees to indemnify, defend, and hold harmless Licensor and TJU and  
9 their respective directors, officers, staff and agents, and in the case of  
11 TJU, trustees, from all liabilities, demands, damages, expenses and  
13 losses (including reasonable attorneys' fees) arising out of or in  
15 connection with this Agreement (collectively, the "Indemnified  
17 Losses"), including without limitation Indemnified Losses resulting  
19 from any use, sale, or other disposition of Licensed Product by  
21 Licensee and any claim that Licensee's use, sale, or other disposition  
of Licensed Product infringes or violates any patent or other  
intellectual property rights. The indemnification rights contained  
herein are in addition to all rights which Licensor and/or TJU may have  
at law or in equity. Licensee hereby agrees that TJU is entitled to  
enforce this Section 5.2 directly against Licensee.

19  
21 ARTICLE 6-TERM

23 6.1 Term Of Agreement. This Agreement shall become effective as of its  
signature by both parties and payment by Licensee of the sum specified in Section 3.1(a).  
25 All obligations thereunder shall expire at the last to expire of any Patent Right having a valid  
claim which Licensee would infringe by its sale of Licensed Products but for this  
Agreement.

27 6.2 Term of Technology Transfer. The transfer of newly developed technology  
29 as foreseen in Section 2.3 shall cease on the fifth (5th) anniversary of the Effective Date.

31 6.3 Early Termination.

- 33 (a) Licensee shall have the right to terminate this Agreement by at least  
35 sixty (60) days written notice to Licensor effective [not earlier than  
twenty (20) months after the Effective Date] *yes*  
37 (b) Either party may terminate this Agreement with immediate effect by  
39 written notice to the other party, if the other party goes into bankruptcy  
or insolvency, or the other party commits a material breach of any  
41 material obligation under this Agreement and fails to remedy such  
breach within sixty (60) days after notice of such breach.

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1           6.4 Termination by Licensor. This Agreement may be terminated by Licensor  
2 upon thirty (30) days written notice to Licensee, upon a species by species basis, if  
3 Licensee ceases, for ~~12~~ consecutive months, to develop or sell Licensed Products within  
4 such species. *after the year 2003.*

5           6.5 Effects of Termination. Termination of this Agreement shall not affect the  
6 continued enforceability of Sections \_\_\_\_\_ and the continued existence of the license back  
7 under Section 2.3 of improvements for the life of the Intellectual Property Right. [Concept  
8 of 20 years does not seem to make sense.]

11  
12  
13                                   **ARTICLE 7-OTHER AGREEMENTS**

14           7.1 Supply of Chimera. In order to ensure the quality of the Chimera, Licensor  
15 shall supply or arrange for supply of Chimera to Licensee at an initial price of one thousand  
16 dollars (US\$1,000) per micromole unpurified and one thousand two hundred dollars  
17 (US\$1,200) per micromole (HPLC purified). Such prices shall not be increased except to  
18 the extent of increases in Licensor's costs. Payments shall be made net thirty (30) days  
19 from delivery. Licensee shall be required to provide forecasts of its requirements for  
20 Chimera. Licensee shall not be required to purchase Chimera in any country where such  
21 a requirement would be illegal or render unenforceable Licensor's Patent Rights.

22           7.2 Committee. *on equal number of* A committee comprising representatives of each party shall be  
23 formed (the "Committee") and shall meet not less than once each calendar quarter to  
24 discuss research, progress toward commercialization and commercialization of Licensed  
25 Products. Prior to each such meeting, Licensee shall provide Licensor with a written report  
26 setting out research conducted and summarizing progress toward commercialization and,  
27 when Licensed Products are available for commercial sale, a summary of marketing and  
28 sales figures and trends. The Committee shall also discuss joint opportunities for licensing  
29 of technology for Plants outside Licensee's Field-of-Use.

30           7.3 Diligence. Licensee shall use reasonable commercial efforts to develop and  
31 commercialize the Licensed Products.

32           7.4 Research. Licensee shall be primarily responsible for development and  
33 execution of research. Licensor shall provide reasonable access to its consultants and  
34 employees to assist Licensee with the technology at a fee of \$2,000 per full day of services  
35 plus reasonable expenses. The parties may agree to undertake joint research, in which  
36 case the parties shall negotiate in good faith to agree to the terms upon which such  
37 research shall be conducted and a research plan. [During the first [three years after the  
38 Effective Date,] Licensor shall provide Licensee with up to five (5) hours per year of  
39 Dr. Kmiec's consulting time per year at two thousand dollars (US\$2,000) per day and

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*Need to disclose ABD/PE agreement  
& that research may be done in  
the field*

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*SPATJ Dr. Kumar & Ben Bowen  
to discuss time  
& process - I  
will expedite*

1 up to twenty (20) hours per year of Dr. Kumar's consulting time at no cost to  
3 Licensee, and up to twenty (20) hours per year at two thousand dollars (US\$2,000)  
per day.]

5 7.5 Trademarks and Use of Names. If Licensee develops a product for  
7 commercialization, the Committee shall consider in good faith whether use of Licensors  
trademarks or a description of Chimera technology in marketing brochures, literature and  
9 labeling for any Licensed Product is appropriate and desirable, and the appropriate  
payment and other terms for such trademark use. Neither party shall use the name of the  
11 other, nor shall Licensee use the name of TJU, or the names of any of their respective staff  
members, employees or students or any adaptation thereof in any advertising, promotional  
13 or sales literature to the extent such use might imply a relationship between the parties, or  
endorsement by either party or TJU of any act or thing or of any product or method  
15 described in such material, without the prior written consent of the other party and TJU  
where applicable, which consent shall not be unreasonably withheld.

17 7.6 Confidentiality. Each party shall:

- 19 (a) maintain the Confidential Information of the other party in confidence  
21 and refrain from disclosing any part of such Confidential Information  
23 to any person or entity other than to its employees and sublicensees  
whose duties or rights justify the need to know such Confidential  
Information;
- 25 (b) not to make any use of the Confidential Information other than for the  
27 purpose of carrying out duties and obligations under this Agreement;
- 29 (c) to take all reasonable steps to protect the Confidential Information  
against disclosure, misuse, loss and theft, which steps include the  
31 execution by all such persons of written agreements containing  
obligations of confidentiality, restricted disclosure and limited use  
33 relative thereto consistent with this Section 7.6 prior to disclosure of  
Confidential Information to them; and
- 35 (d) in the event that a third party wishes to evaluate Confidential  
37 Information in connection with a proposed business transaction with  
a party, disclose only as much of the Confidential Information to that  
39 third party as is necessary to conduct such evaluation, provided that  
prior to disclosure such third party executes a written agreement  
41 prohibiting use of the Confidential Information for any reason other  
than evaluation of such transactions and containing obligations of  
43 confidentiality consistent with this Section 7.6.

1           7.7 Press Releases. Prior to any press release concerning this Agreement, both  
3 parties shall agree on the content, such consent not to be unreasonably withheld.

5           7.8 Regulatory Approvals. The parties shall provide to one another (at no cost)  
7 all materials, data and information in their possession needed to seek and obtain  
9 regulatory approvals necessary for the use and sale of products which use the Licensed  
Technology. Neither party shall use any regulatory information and/or packages developed  
by the other for the benefit of a third party.

## 11                                   **ARTICLE 8--MISCELLANEOUS PROVISIONS**

13           8.1 Force Majeure. Neither party shall be liable for failure to perform its  
15 obligations hereunder for so long as that failure may be the result of an event beyond its  
reasonable control (a "force majeure" event), provided that such party uses all reasonable  
17 efforts to comply with the terms of this Agreement to the extent that it is able to do so.

19           8.2 Entire Agreement. This Agreement, together with all Exhibits attached hereto,  
21 constitutes the entire Agreement between the parties with respect to the present subject  
matter, all prior negotiations, agreements and understandings being expressly canceled  
hereby.

23           8.3 Amendment. This Agreement may be amended only by a written agreement  
25 embodying the full terms of the amendment signed by authorized representatives of both  
parties.

27           8.4 Assignment. Neither party may assign their rights or obligations under this  
29 Agreement without prior written approval from the other party, except as part of the sale or  
transfer of substantially all the business to which this Agreement pertains, provided that  
31 such purchaser expressly agrees to assume that party's rights and obligations under this  
Agreement.

33           8.5 Severability. Should any provision of this Agreement be illegal, invalid or  
35 unenforceable under applicable law, the remaining provisions of this Agreement shall be  
construed as if such illegal, invalid or unenforceable provision had not been contained  
37 herein. The parties shall attempt to negotiate a provision replacing such provision and  
providing comparable benefits to each party, but in the event that such negotiations do not  
39 result in agreement within ninety (90) days, either party shall have the right to terminate this  
Agreement by ninety (90) days written notice to the other party.

41           8.6 No Strict Construction. The language used in this Agreement shall be  
deemed to be the language chosen by both parties hereto to express their mutual intent

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1 and no rule of strict construction against either party shall apply to any term or condition of  
3 this Agreement.

5 8.7 Relationship of Parties. Nothing contained in this Agreement shall be  
7 construed as creating a partnership, joint venture, agency or an association of any kind.

9 8.8 No Waiver. The failure of one party hereto to enforce at any time any of the  
11 provisions of this Agreement, or any rights in respect thereto, or to exercise any election  
13 herein provided, shall in no way be considered to be a waiver of such provision, rights or  
15 elections or in any way to affect the validity of this Agreement. Any waiver must be in  
17 writing.

19 8.9 Interpretation. The headings contained in this Agreement are for convenience  
21 only and shall not affect the interpretation of this Agreement. In this Agreement, the word  
23 "including" shall be deemed to be followed by "without limitation", the words "hereof" and  
25 "herein" and "hereunder" refer to this Agreement as a whole, and the singular includes the  
27 plural and vice versa.

29 8.10 Notices. Notices shall be given by first class mail, by Federal Express or  
31 other recognized courier requiring signature on receipt, or by telecopy confirmed by  
33 contemporaneous phone conversation with the recipient of the telecopy, and shall be  
35 addressed to the other party at the address set forth below (or at such address as a party  
37 may specify by notice to the other):

39 If to Licensee: Pioneer Hi-Bred International, Inc.

41 Attention: \_\_\_\_\_  
43 700 Capitol Square  
45 400 Locust Street  
47 Des Moines, IA 50309-2340  
49 Telephone: (515) 248-4800  
51 Telecopy: (515) 253-2478

53 If to Licensor: Kimeragen, Inc.

55 Attention: \_\_\_\_\_  
57 300, Pheasant Run  
59 Newtown, PA 18940  
61 Telephone: (215) 504-4444  
63 Telecopy: (215) 504-4545

65 8.11 Governing Law. This Agreement shall be governed by and construed in  
67 accordance with the laws of [New York] without giving effect to any choice of law or conflict  
69

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*Pennsylvania*

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1 of law provision or rule that would cause the application of the laws of any jurisdiction other  
3 than [New York].

5 8.12 Counterparts. This Agreement may be executed in one or more counterparts,  
7 each of which shall be deemed an original, but all of which together shall constitute one and  
the same instrument.

9 8.13 Dispute Resolution. The parties shall work together to remedy any difficulties  
11 which may arise in connection with this Agreement. All disputes arising out of this  
13 Agreement shall be referred to decision forthwith to a senior executive of each party who  
15 is, if possible, not involved in the dispute. If no agreement can be reached through this  
17 process within thirty (30) days of request by one party to the other to nominate a senior  
19 executive for dispute resolution, then either party hereto shall be entitled to refer such  
dispute to three arbitrators for arbitration, such arbitration to be held in Chicago, Illinois on  
an expedited basis in accordance with the rules and regulations of the American Arbitration  
Association. One arbitrator shall be appointed by each party within thirty (30) days of a  
request for arbitration or receipt of notice thereof, with such arbitrators to appoint the third  
arbitrator within thirty (30) days of the appointment of the latter of the party arbitrators. The  
decision of the arbitrators shall be irrevocable and fully accepted by both parties.

21 IN WITNESS WHEREOF, the parties have caused their duly authorized  
23 representative to execute this Agreement as of the Effective Date.

25 PIONEER HI-BRED INTERNATIONAL, INC.

27 By: \_\_\_\_\_

29 Name: \_\_\_\_\_

31 Title: \_\_\_\_\_

33 KIMERAGEN, INC.

35 By: \_\_\_\_\_

37 Name: \_\_\_\_\_

39 Title: \_\_\_\_\_

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**EXHIBIT A**

**Patent Rights**

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201 543 7670:#15/16

K & E NEW YORK-

8-31-96 : 6:15PM :

SENT BY :



**EXHIBIT B**

**Royalty Calculations**

*Peter Zoller  
calculating*

	ROYALTY	MAXIMUM ROYALTY*
Corn (80K unit)	\$0.250	\$0.50
Sorghum (50# unit)	\$0.134	\$0.268
Soybean (50# unit)	\$0.043	\$0.087
Sunflower (200K unit)	\$0.280	\$0.560
Canola (????)	<del>[\$0.000]</del>	<del>[\$0.000]</del>
Alfalfa (50# unit)	\$0.462	\$0.924

\* For more than one use of the Licensor Technology.

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**KIRKLAND & ELLIS**

PARTNERSHIPS INCLUDING PROFESSIONAL CORPORATIONS

Rosemary T. Langford  
To Call Writer Direct:  
212 446-4946

Citycorp Center  
153 East 53rd Street  
New York, New York 10022-4875  
212 446-4800

Facsimile:  
212 446-4900

September 11, 1996

VIA FACSIMILEPRIVILEGED & CONFIDENTIAL  
ATTORNEY CLIENT COMMUNICATION

Gerald L. Messerschmidt, M.D., F.A.C.P.  
President & CEO  
Kimeragen, Inc.  
284 Lafayette Lane  
Wayne, PA 19087

Dr. Ramesh Kumar  
Vice President Technology &  
Product Development  
Kimeragen, Inc.  
60 Yard Road  
Pennington, NJ 08534

Re: Cornell

Gentlemen:

Pioneer

1. The paragraph of the Cornell license dealing with sublicensing (Section XII, page 15) is strange. It states that royalty payments "as above" shall be made by sublicensee. This seems to suggest that sublicensees have to be charged the same (percentage) royalty as Kimeragen. However, this is unusual and the logic of the language on page 10 relating to sublicenses is that Cornell just receives a percentage of the royalty received by Kimeragen and shouldn't care about the royalty calculation in the sublicense. However, I believe that we should confirm with Cornell that we can charge Pioneer on a per day basis.

2. We should explore whether Cornell has any problem with the concept of a sublicensee (Pioneer) further sublicensing. TJU did not.

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Chicago

London

Los Angeles

Washington, D.C.

# KIRKLAND & ELLIS

PARTNERSHIPS INCLUDING PROFESSIONAL CORPORATIONS

Stephen P. H. Johnson  
To Call Writer Direct:  
212 446-4920

Citicorp Center  
153 East 53rd Street  
New York, New York 10022-4675

212 446-4800

Facsimile:  
212 446-4900

September 10, 1996

## VIA FACSIMILE

Peter A. Fuller, Ph.D.  
Director, Technology Discovery and Assessment  
Michael E. Yates, Esq.  
Patent Counsel  
Pioneer  
700 Capital Square  
400 Locust Street  
Des Moines, Iowa 50309

Re: Kimeragen License

Gentlemen:

I enclose a draft of the license between Kimeragen and Pioneer. We had planned to get this draft out a week ago, but unfortunately Bo is quite ill at the moment.

Please note that this draft has not been seen by our client in its final form. However, we did want to move matters forward to try and make up for the difficulties experienced last week. Let me know if it would expedite your review to have a copy on diskette or e-mailed.

Very truly yours,



Stephen P.H. Johnson

cc: Leonard Shakin (w/enclosure)  
Gerald Messerschmidt (w/enclosure)  
Bo Allen (w/enclosure)

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Chicago

London

Los Angeles

Washington, D.C.

# Facsimile Cover Sheet

Number of pages including cover sheet:

**Message:**

**Bo & Stephen,**

For your review and comment. Thanks again for your cooperation.

PAF

Hard Copies & Disk Have Been  
Sent via overnight carriers for  
monday delivery -  
DAF

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2  
4 Draft 9/10/96 Kimeragen  
Draft 9/20/96 Pioneer

"Red-line"

6  
8 LICENSE AGREEMENT

10 THIS LICENSE AGREEMENT (the "Agreement") is made and entered into this \_\_\_\_ day of  
12 \_\_\_\_\_, 1996 (the "Effective Date") between Pioneer Hi-Bred International, Inc., an Iowa  
corporation of 700 Capitol Square, 400 Locust Street, Des Moines, IA 50309-2340 ("Licensee"),  
and Kimeragen, Inc., a Delaware corporation, of 300 Pheasant Run, Newtown, PA 18940  
("Licensor").

14 WHEREAS, Licensor is the exclusive licensee of Thomas Jefferson University ("TJU")  
under U.S. and foreign patent applications with respect to a chimeric vector for application in gene  
16 therapy developed by TJU and certain methods and processes using that chimeric vector; and

18 WHEREAS, Licensee wishes to obtain an exclusive, worldwide license to use the  
20 technology to make and sell products~~under such technology to use and sell certain Licensed~~  
~~Products~~ in the Licensee Field-of-Use (as defined below).

22 NOW THEREFORE, in consideration of the mutual promises and covenants set forth herein  
and for good and valuable consideration, the adequacy and sufficiency of which is hereby  
24 acknowledged, the parties hereby agree as follows:

26  
28 ARTICLE 1--DEFINITIONS

30 1.1 "Affiliate" shall mean any company or entity related to Licensee or to a Subsidiary of  
32 Licensee by contract and through which Licensee or Subsidiary of Licensee produces and/or  
markets seed to end-user customers, but only for the period or periods during which such a  
contractual relationship exists.

34 1.2 "Chimera" shall mean the chimeric vector of RNA and DNA as claimed in the Patent  
Rights or otherwise the subject of non-patented Licensor Technology.

36 1.3 "Confidential Information" shall mean all information, in whatever form, which is  
38 disclosed by either party prior to or subsequent to the Effective Date of this Agreement ~~and which~~  
~~relates in any way to the Licensor Technology.~~

40 1.4 "Intellectual Property" shall mean without limitation, all filed patents, patentable  
42 inventions, ~~plant varieties~~, know-how, trade secrets, techniques and ideas and all technical material  
44 which further includes ~~germplasm, cell lines, plants, seeds,~~ DNA and RNA sequences and genes,  
oligos and proteins, and associated methods and all applications therefor.

46 1.5 "Kimeragen Licensors" shall mean TJU and the Cornell Research Foundation, Inc.  
48 ("Cornell").

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1.6 "Licensed Products" shall mean products containing genetic material that has been altered (in that generation or an earlier generation) by the use of the Licenser Technology.

1.7 "Licensee Field-of-Use" shall mean ~~Product Use and Research Use, excluding Pharmaceutical Use, in Corn, Sunflower, Soybean, Canola/Rape, Alfalfa, Tobacco, Arabadopsis and Sorghum and Research Use excluding Pharmaceutical Use, in Tobacco and [Arabadopsis?],~~ provided that no Tobacco or ~~Arabadopsis~~ products related in any way to or any product products produced by or from use of the Licenser Technology such Research Use shall be sold by Licensee.

1.8 "Licensee Technology" shall mean all Intellectual Property conceived, controlled by, and/or developed by Licensee pertaining to the Chimera and associated methods required for its use.

1.9 "Licenser Field-of-Use" shall mean all uses outside the Licensee Field-of-Use.

1.10 "Licenser Technology" shall mean the Chimera, all Intellectual Property conceived, controlled by, and/or developed by Licenser covering the Chimera and other Intellectual Property of Licenser (including Rec2 gene (as defined below)) but use of any such Intellectual Property shall be limited to the extent required for the use of the Chimera in the Licensee Field-of-Use for Product Use. Licenser Technology shall further include all improvements to Licenser Technology.

1.11 "Patent Rights" shall mean patents and patent applications and all foreign counterparts thereof in the Territory as listed in Exhibit A, including all future divisionals and continuations, continuations in part, additions, confirmations, renewals, extensions and reissues of patents and patent applications and their equivalents covering the Licenser Technology.

1.12 "Plants" shall mean multicellular rooted organisms containing chlorophyll and cellulose cell walls.

1.13 "Pharmaceutical Use" shall mean all uses related to pharmaceutical compounds that are or would be regulated by the FDA Centers of Biological Evaluation and Research (CBER), Drug Evaluation and Research (CDER), Devices and Radiologic Health (CDRH) and Veterinary Medicine (CVM) in any species, and all similar foreign agencies.

~~1.14 "Product Use" shall mean use of the Licenser Technology where the actual product sold does contain genetic material that has been altered (in that generation or an earlier generation) by the use of the Licenser Technology.~~

1.15 "Rec2" shall mean that gene as described in relation to *Ustilago* in U.S. patent application serial no. 08/373,134 ("*Ustilago* Rec2") and in relation to humans in patent disclosure Pennie & Edmonds docket no. 79910010 ("Human Rec2").

~~1.16 "Research Use" shall mean use of the Licenser Technology in the Licensee Field of Use where no products sold contain genetic material that has been altered by the use of the Licenser Technology.~~

1.175 "Sublicensee" shall mean any company or entity which is sublicensed by Licensee pursuant to the terms of Section 2.2.

1.186 "Subsidiary" shall mean any company or entity at least 40% ~~50%~~ of whose voting stock is owned or directly or indirectly controlled by Licensee.

1.197 "Territory" shall mean the world.

## ARTICLE 2--LICENSE

2.1 Grant of License. Licensors hereby grants to Licensee, upon the terms and conditions set forth herein, a royalty-bearing, exclusive license under the Licensors Technology, including the Patent Rights, to use and make (pursuant to section 7.2) Chimera for research purposes and ~~to (a) use and have used Chimera for Research Use, and (b) use and have used Chimera for Product Use, in both cases to the extent necessary to~~ develop and commercialize Licensed Products in the Licensee Field-of-Use in the Territory

(a) Licensee shall have the right to assign within the Licensee Field-of-Use or Territory (or part thereof) the license granted in this Section 2.1 to certain wholly-owned Subsidiaries; and

(b) Licensee shall have the right to subcontract to Licensee's Subsidiaries and Affiliates activities relating to the growing and marketing of Licensed Products within the Licensee Field-of-Use or Territory (or part thereof) for the purposes of conducting Licensee's ongoing business.

2.2 Such Notwithstanding any such assignment as per (a) above or subcontracting as per (b) above, shall not be considered a sublicense, provided that Licensee shall be responsible for the activities, including all reporting and royalty obligations, of such Subsidiaries and Affiliates as if they were the part of Licensee.

2.23 Sublicensing. Licensee may grant sublicenses within the scope of the licenses granted in Section 2.1 to third parties, provided that:

(a) Licensors ~~has shall have~~ the right pursuant to any applicable contract between Licensors and a third party (or third parties) to allow Licensee to sublicense the Intellectual Property right in question;

(b) Licensee shall cause all such Sublicensees ~~sublicensees~~ to comply with the provisions of this Agreement in the same manner as Licensee is bound hereby and shall contain provisions protecting the Kimeragen Licensors as specified on Exhibit B;

(c) No Sublicensee ~~sublicensee~~ shall have the further right to sublicense;

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(d) Licensee shall be responsible for compliance by such Sublicensees ~~sublicensees~~ with the terms of this Agreement; and

(e) Licensee shall give the Licenser prompt written notice of all sublicenses granted pursuant to this Section 2.2 as well as copy of the sublicense agreement.

2.34 Improvements. During the term provided in Section 6.2, the parties shall promptly disclose to each other in reasonable detail any newly developed Intellectual Property which is related to the Licenser Technology or Licensee Technology.

(a) Licensee hereby grants to Licenser an exclusive paid up worldwide license to the Licensee Technology, with the right to grant sublicenses (which may in turn include the right to grant further sublicenses), for all uses and applications in the Licenser Field-of-Use, provide-provided that Licenser shall have no right to Licensee Technology for any uses and applications in Plants. The terms and conditions of such license shall be substantially like those contained in this present license recognizing that the parties to the new license are reversed.

~~Licenser shall license Licensee to use improvements in Licenser Technology pursuant to Section 2.1. During the term provided in Section 6.2, the parties shall promptly disclose to each other in reasonable detail any inventions or improvements which relate to the Licenser Technology or Licensee Technology.~~

2.45 Limitations on Exclusivity. Except as provided below, the licenses of Technology granted under Sections 2.1 and 2.3 shall be exclusive in the Territory in the respective field of use of each party.

~~(a) The license shall be non-exclusive to the extent that rights of either party in the future shall be non-exclusive;~~

~~(ba)~~ The licenses shall be non-exclusive to the extent required by government regulations; and

~~(c) The licenses shall be non-exclusive to the extent of research use.~~

2.56 Freedom to Use. In the event that either party shall license third-party Intellectual Property to gain freedom-to-operate in that party's Field-of-Use and such Intellectual Property is also needed by the other party to gain freedom-to-operate in its Field-of-Use, subject to Section 3.6, the first party shall use all reasonable efforts to license such Intellectual Property so that other party's use of Licenser such Intellectual Property is covered. Neither party shall enter into any agreement that would preclude the other party from being able to also obtain a license.

2.67 Protection of Technology. Licensee shall not use the Licenser Technology for any purpose other than to ~~make, use and sell Licensed Products,~~ as provided in this Agreement. Licensee shall take no action in respect of the Licenser Technology which is inconsistent with the terms of the license granted under this Agreement.



2        **2.78 Acknowledgment of Rights.** Licensee acknowledges that Licensee's right to use the  
4        Licenser Technology arises only out of the ~~licensee sublicense~~ granted under this Agreement. All  
6        Licensed Products manufactured under issued patents shall bear a patent notice on the label as  
      may be necessary or appropriate under the laws of the country in the Territory in which the  
      Licensed Product is sold as reasonably specified by Licenser to Licensee.

8        ~~2.8 Reservation of Rights. This Agreement contains no grants under any Intellectual~~  
10        ~~Property of Licenser, except to the extent of those explicitly granted under Section 2.1. Without~~  
12        ~~limitation, no right is granted to sequence Rec2 or any related gene or recombinase in Plants. Any~~  
      ~~such work shall be carried out as a joint research project pursuant to Section 7.4 with Licenser and~~  
      ~~Licensee jointly owning any genes isolated as a result of such work.~~

### ARTICLE 3--PAYMENT

16        **3.1 Payment.** In consideration of the rights granted herein, the Licensee shall pay to the  
18        Licenser:

20            (a)    three million dollars (US\$3,000,000) upon the date of execution of this  
22            Agreement, provided that this Agreement shall not become effective until  
24            such payment is shall be made. Such payment shall be for the use of the  
      rights granted herein for a period extending from the Effective date to twenty  
      (20) months after the Effective Date.

26            (b)    two million dollars (US\$2,000,000) twenty (20) months after the Effective  
28            Date provided that Licensee shall not have issued a notice of termination  
30            pursuant to Section 6.3(a) effective on or before such date. Such payment  
32            shall be comprised of two portions: i) one million nine hundred thousand  
34            dollars (\$1,900,000) for patents and patent applications in the Patent Rights  
      that are filed after June 8, 1995 or issued before twenty months after the  
      Effective Date, and ii) one hundred thousand dollars (\$100,000) for patent  
      applications and patents in the Patent Rights but not included in i) above.

36            (c)    a royalty per unit bag of Licensed Product as specified on Exhibit C.

38            (d)    ~~fifty percent (50%) of the value of all sublicense a portion of sublicensing fees~~  
40            ~~and/or royalties revenues (including fees and royalties) as provided in~~  
      Section 3.3.

42        **3.2 Royalty Conditions.** Royalties shall:

44            (a)    ~~be payable only on the sales of Licensed Product in countries where pending~~  
46            ~~or issued patents or plant variety protection exists covering the Chimera or~~  
      ~~Licensed Products or on sales of Licensed Product where the Licensed~~  
      ~~Product was developed or grown in countries where such rights exist; be~~

payable on the sale by Licensee (including Licensee's Affiliates, Subsidiaries or sublicensees) of Licensed Products to end-use customers, but only when such Licensed Products' sale, development or production is, at the time of sale covered by an issued Patent Right in the country where Licensed Product is sold, developed or produced;

- (b) accrue upon the sale of Licensed Product (including Affiliates and Subsidiaries) as determined by U.S. GAAP~~recognized by the SEC (please explain)~~;
- (c) be due and payable in U.S. dollars annually within sixty (60) days after the close of Licensee's fiscal year (August 31);
- (d) not be due and payable on i) Licensed Products used for Licensee's internal purposes (test beds) but shall be due on samples provided to end-use customers and Affiliates, or ii) sales of Licensed Product to or among Licensee and Licensee's Affiliates, Subsidiaries or Sublicensees;
- (e) be prorated based on the package sizes shown on Exhibit C for package sizes not shown on Exhibit C;
- (f) be due and payable on Licensed Products used by Licensee for the production of commercial grain at the same royalty amount as if the Licensed Products were sold.

3.3 Sublicenses. Licensor shall be entitled to share in all sublicensing fees and royalties on a 50:50 basis. In the event that Licensee barter or trades the rights as granted in Section 2.1 to a third party a Sublicense of Licensor Technology for other technology, or otherwise shall receive non-cash consideration, no fees shall be due to Licensor, ~~to the extent of such non-cash consideration but~~ Licensee shall be obligated to pay royalties to Licensor~~Licensee~~ on sales by such third party the Sublicensee as if such sales were sales by Licensee ~~at but at seventy five percent (75%) of the rate provided on Exhibit C.~~ Licensee shall be responsible for monitoring and reporting on the sales of its Sublicensees and shall be responsible for all royalties due to Licensor.

3.4 Reports. Licensee shall provide royalty reports to Licensor within sixty (60) days after the close of Licensee's fiscal year. Such reports shall contain information about Licensee's sales as well as sales by and revenue received from Sublicensees reasonably necessary to verify royalties due, including:

- (a) A listing of all Licensed Products qualifying for royalties sold by Licensee (including Subsidiaries and Affiliates) and Sublicensees, sorted by individual product, crops ~~species and~~ country;
- (b) Royalty calculation; and
- (c) Royalties due and payable.

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3.5 **Audit.** The Licensee shall keep full and accurate records at its principal place of business in the United States of all Licensed Products grown, distributed, or sold by Licensee (including Subsidiaries and Affiliates) and its Sublicensees during the term of this Agreement. Such records shall be open, at reasonable times, for three (3) years following the end of the fiscal year to which they pertain, to an inspection by a mutually agreed upon Certified Public Accounting Firm, Licensor or its representative, at Licensor's expense, for the purposes of verifying Licensee's royalty payments under this Agreement. If a discrepancy of greater than five percent (5%) is found in any payment due hereunder, Licensee shall reimburse Licensor for the cost of such inspection and promptly pay such overdue amount together with interest at the rate specified in Section 3.7.

3.6 **Third Party Royalties.** In any case where use of the Licensor Technology licensed to Licensee is in the future subject to a royalty (whether lump-sum or payable by reference to sales) to a third party other than Kimeragen Licensors with respect to currently licensed Licensor Technology, then in the event that Licensee determines to use such Licensor Technology, Licensee shall, in addition to the royalty specified in Section 3.1, be responsible for payment to Licensor of a further amount equal to the royalty payable to the third party attributable to sales by Licensee.

3.7 **Late Payments.** All payments to be made by the Licensee to the Licensor hereunder shall bear interest at the prime or equivalent rate as quoted by Citibank N.A., New York, New York, on the day the payment is overdue plus two percent (2%) per annum until paid.

#### **ARTICLE 4--PATENTS**

4.1 **Prosecution.** Licensor shall use reasonable efforts to prosecute the Patent Rights to the extent within the control of Licensor. In the event that Licensor shall elect not to continue prosecution such that the Chimera would not be subject to patent protection, then Licensee shall have the right to assume such prosecution at its expense, but any royalty obligation relating solely thereto shall cease.] [Chimera patent about to issue.]

4.2 **Infringement Actions.** Licensee shall notify Licensor promptly of any action, claim or threat of patent infringement suit, either oral or written, or the commencement of any such patent infringement suit against Licensee relating to the Licensor Technology or of any infringement by a third party of the Patent Rights within the Licensee Field of-Use. The parties shall cooperate in the development and execution of a strategy to defend against such any such action against Licensee or to prevent any such infringement by a third party.

#### **ARTICLE 5--WARRANTIES & INDEMNITIES**

##### **5.1 Warranties.**

- (a) Each party represents and warrants to the other that it has full right, power and authority to enter into this Agreement and that the terms of this Agreement do not conflict with any other contractual obligations it has.

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(b) Licensor represents and warrants that, as of the execution date, it knows of no Intellectual Property that would prevent Licensee from practicing the Licensor Technology.

(c) Licensor represents and warrants that it has the freedom to enter into this Agreement and the right to provide the rights and license contemplated in Section 2.1.

(d) Licensor represents and warrants that, as of the Effective Date, Licensor has disclosed to Licensee all known, and knows of no other, royalty obligations pursuant to Section 3.6;

(de). No Other Warranties. Licensor makes no other warranty or representation with respect to the Licensor Technology, nor is Licensor in any way responsible for the utility of any Licensor Technology. LICENSOR HEREBY EXPRESSLY DISCLAIMS ANY AND ALL WARRANTIES AND REPRESENTATIONS, EXPRESS OR IMPLIED, ARISING BY LAW OR CUSTOM, WITH RESPECT TO THE LICENSOR TECHNOLOGY, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, PATENTABILITY OR NON-INFRINGEMENT. LICENSOR DOES NOT IN ANY WAY PROMISE THAT THE LICENSOR TECHNOLOGY SHALL PRODUCE ANY PARTICULAR RESULTS, PRODUCTS OR PROFITABILITY.

5.2 Indemnities. Licensee hereby waives any claim against Licensor and the Kimeragen Licensors and agrees to indemnify, defend, and hold harmless Licensor and the Kimeragen Licensors and their respective directors, officers, employees and agents, and in the case of the Kimeragen Licensors, their trustees, officers, agents and employees and those of any associated university, from all liabilities, demands, damages, expenses and losses (including without limitation for death, personal injury, illness or property damage, and including reasonable attorneys' fees) arising out of or in connection with this Agreement (collectively, the "Indemnified Losses"), including without limitation Indemnified Losses resulting from any exercise or use by Licensee or its transferees of Patent Rights, and any use, sale, or other disposition of Licensed Product by Licensee or its transferees and any claim that Licensee's use, sale, or other disposition of Licensed Product infringes or violates any patent or other intellectual property rights. The indemnification rights contained herein are in addition to all rights which Licensor and/or the Kimeragen Licensors may have at law or in equity. Licensee hereby agrees that the Kimeragen Licensors are entitled to enforce this Section 5.2 directly against Licensee. Licensee shall cause its Affiliates and Subsidiaries, contractors and sub-contractors to waive claims against and indemnify the Kimeragen Licensors on the terms set forth above. Notwithstanding the foregoing, the indemnities provided by Licensee herein shall not apply to the extent the indemnified losses would be or are covered by the foregoing Licensor's warranties.

#### **ARTICLE 6--TERM**

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6.1 Term Of Agreement. This Agreement shall become effective as of its signature by both parties and payment by Licensee of the sum specified in Section 3.1(a). All rights and obligations hereunder shall expire at the last to expire of any Patent Right having a valid claim which Licensee would infringe by its sale of Licensed Products but for this Agreement.

6.2 Term of Technology Transfer. The transfer of improvements ~~newly developed technology~~ as foreseen in Section 2.3 shall cease on the eight (8<sup>th</sup>) ~~fifth (5th)~~ anniversary of the Effective Date.

6.3 Early Termination.

(a) Licensee shall have the right to terminate this Agreement by at least sixty (60) days written notice to Licensor ~~effective not earlier than twenty (20) months after the Effective Date.~~

(b) Either party may terminate this Agreement with immediate effect by written notice to the other party, if the other party goes into bankruptcy or insolvency, or the other party commits a material breach of any material obligation under this Agreement and fails to remedy such breach within sixty (60) days after notice of such breach.

6.4 Restriction of Licensee Field-of-use ~~Termination by Licensor.~~ Licensor shall be entitled to modify Licensee Field-of-Use on a crop-by-crop basis. ~~This Agreement may be terminated by Licensor upon thirty (30) days written notice to Licensee, upon a species by species basis, if Licensee ceases, for twelve (12) consecutive months, after the year 2003, to develop or sell Licensed Products within such species.~~

6.5 Effects of Termination. Termination of this Agreement shall not affect the continued enforceability of Sections ~~\_\_\_\_\_ and the continued existence of the license back under Section 2.3 of improvements for the life of the Intellectual Property Right. [Concept of 20 years does not seem to make sense.]~~

**ARTICLE 7--OTHER AGREEMENTS**

7.1 Supply of Chimera. In order to ensure the quality of the Chimera, Licensor shall supply or arrange for supply of Chimera to Licensee at an initial price of one thousand dollars (US\$1,000) per micromole unpurified and one thousand two hundred dollars (US\$1,200) per micromole (HPLC purified). Such prices shall not be increased except to the extent of increases in Licensor's costs. Licensor shall make a reasonable effort to supply the agreed upon quantity of Chimera within fifteen (15) days of receipt of the order. Payments shall be made net thirty (30) days from delivery. Licensee shall be required to provide forecasts of its requirements for Chimera.

7.2 Obligation to Purchase of Chimera. Upon the second anniversary of the Effective date, Licensee may make internally or have made or buy from another vendor Chimera for its own internal use. Licensee shall not be required to purchase Chimera in any country where such a requirement would be illegal or render unenforceable Licensor's Patent Rights.

2           **7.23 Committee.** A committee comprising an equal number of representatives of each  
3 party shall be formed (the "Committee") and shall meet not less than once each calendar year  
4 quarter to discuss research, progress toward commercialization and commercialization of Licensed  
5 Products. Licensee shall provide a research plan with benchmarks for development and  
6 commercialization of Licensed Products in each ~~crop~~species within the Licensee Field-of-Use.  
7 Prior to each such meeting, Licensee shall provide Licensor with a written report setting out  
8 research conducted measured against benchmarks, and summarizing progress toward  
9 commercialization and, when Licensed Products are available for commercial sale, a summary of  
10 marketing and sales figures and trends. The Committee shall also discuss joint opportunities for  
11 licensing of technology for Plants outside Licensee Field-of-Use.

12           **7.34 Diligence.** Licensee shall use reasonable commercial efforts to develop and  
13 commercialize the Licensed Products.

14           **7.45 Research.** Licensee shall be primarily responsible for development and execution of  
15 research in Licensee Field-of-Use. Licensor shall provide reasonable access to its consultants and  
16 employees to assist Licensee with the technology at a fee of \$2,000 per full day of services plus  
17 reasonable expenses. The parties may agree to undertake joint research, in which case the parties  
18 shall negotiate in good faith to agree to the terms upon which such research shall be conducted  
19 and a research plan. [During the first [three years after the Effective Date,] Licensor shall  
20 provide Licensee with up to five (5) days per year of Dr. Kmiec's consulting time per year at  
21 two thousand dollars (US\$2,000) per day and up to twenty (20) days per year of Dr. Kumar's  
22 (or other Licensor's scientists') consulting time at no cost to Licensee, and up to twenty (20)  
23 hours per year at two thousand dollars (US\$2,000) per day.] [Subject to review]

24           **7.66 Trademarks and Use of Names.** If Licensee develops a Licensed Product product for  
25 commercialization, the Committee shall consider in good faith whether use of Licensor's  
26 trademarks or a description of Chimera technology in marketing brochures, literature and labeling  
27 for any Licensed Product is appropriate and desirable, and the appropriate payment and other  
28 terms for such trademark use. Neither party shall use the name of the other, nor shall Licensee  
29 use the name of any Kimeragen Licensor, or the names of any of their respective staff members,  
30 employees or students or any adaptation thereof in any advertising, promotional or sales literature  
31 to the extent such use might imply a relationship between the parties, or endorsement by either  
32 party or any Kimeragen Licensor of any act or thing or any product or method described in such  
33 material, without the prior written consent of the other party and the Kimeragen Licensor where  
34 applicable, which consent shall not be unreasonably withheld.

35           **7.67 Confidentiality.** Each party shall:

- 36           (a) maintain the Confidential Information of the other party in confidence and  
37 refrain from disclosing any part of such Confidential Information to any  
38 person or entity other than to its employees, consultants and sublicensees  
39 whose duties or rights justify the need to know such Confidential Information;
- 40           (b) not to make any use of the Confidential Information other than for the  
41 purpose of carrying out duties and obligations under this Agreement;

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(c) to take all reasonable steps to protect the Confidential Information against disclosure, misuse, loss and theft, which steps include the execution by all such persons of written agreements containing obligations of confidentiality, restricted disclosure and limited use relative thereto consistent with this Section 7.6 prior to disclosure of Confidential Information to them; and

(d) in the event that a third party wishes to evaluate Confidential Information in connection with a proposed business transaction with a party, disclose only as much of the Confidential Information to that third party as is necessary to conduct such evaluation, provided that prior to disclosure such third party executes a written agreement prohibiting use of the Confidential Information for any reason other than evaluation of such transactions and containing obligations of confidentiality consistent with this Section 7.6.

The provisions of this Section 7.6 shall not apply to any part of the Confidential Information disclosed by one party to the other (a) which is agreed in writing by the disclosing party to be excluded; or (b) which the receiving party can show was known to or developed by it prior to Confidential Information first being received by it from, or disclosed to it by, the disclosing party; or (c) which is public knowledge, or becomes public knowledge in the future, other than through acts or omissions of the receiving party in breach of this Agreement; or (d) which is lawfully obtained by receiving party from sources independent of the disclosing party who have a lawful right to possess and disclose such Information; or (e) which it is necessary for the receiving party to disclose in order to comply with any applicable law or if required to do so by order of any court or any other judicial or administrative body, provided that prior to making such disclosure the receiving party gives the disclosing party notice of the requirement of disclosure and the information to be disclosed.

7.78 Press Releases. Prior to any press release concerning this Agreement, both parties shall agree on the content and timing, such consent not to be unreasonably withheld.

7.89 Regulatory Approvals. The parties shall provide to one another (at no cost) all materials, data and information in their possession needed to seek and obtain regulatory approvals necessary for the use and sale of products in their respective Fields-of-Use which use the Licensed Technology. Neither party shall use any regulatory information and/or packages developed by the other for the benefit of a third party. Licensee shall comply with all regulatory requirements relating to the Licensed Products and shall take all reasonable or required steps to ensure that the Licensed Products are safe and lawful.

7.910 Product Liability Insurance. Licensee shall obtain and maintain commercial general liability insurance, including commercial liability, product liability and completed operations insurance coverage in a minimum amount of five million dollars (\$5,000,000) per loss including coverage for contractual liability. Licensor and the Kimeragen Licensors and their respective officers, directors, trustees, members of governing boards and employees will be named insureds under all such insurance. Such insurance shall also provide that Licensor and the Kimeragen Licensors be given notice of any modification thereof and at least ten (10) days prior written notice of cancellation or termination and the reason therefor. A certificate of insurance evidencing such

coverage will be provided to Licensor and the Kimeragen Licensors and, upon each annual anniversary of this Agreement, Licensee shall provide written confirmation issued by the insurer or an independent insurance agent confirming that insurance is maintained in accordance with the above requirements. At Licensee's sole determination, Licensee may elect to be self-insured in accordance with reasonable business practices.

7.11 Upon request from Licensee, Licensor shall diligently undertake to have this licensing agreement registered by the competent authorities of the countries of the Territory in order to safeguard Licensee's ability to join Licensor in any Intellectual Property rights enforcement action brought by Licensor against a third party and allow Licensee to claim damages resulting from the violation of the Intellectual Property rights licensed to him.

#### ARTICLE 8--MISCELLANEOUS PROVISIONS

8.1 Force Majeure. Neither party shall be liable for failure to perform its obligations hereunder for so long as that failure may be the result of an event beyond its reasonable control (a "force majeure" event), provided that such party uses all reasonable efforts to comply with the terms of this Agreement to the extent that it is able to do so.

8.2 Entire Agreement. This Agreement, together with all Exhibits attached hereto, constitutes the entire Agreement between the parties with respect to the present subject matter, all prior negotiations, agreements and understandings being expressly canceled hereby.

8.3 Amendment. This Agreement may be amended only by a written agreement embodying the full terms of the amendment signed by authorized representatives of both parties.

8.4 Assignment. Neither party may assign their rights or obligations under this Agreement without prior written approval from the other party, except as i) as set forth in Article 2 and ii) as part of the sale or transfer of substantially all the business to which this Agreement pertains, provided that such purchaser expressly agrees to assume that party's rights and obligations under this Agreement.

8.5 Severability. Should any provision of this Agreement be illegal, invalid or unenforceable under applicable law, the remaining provisions of this Agreement shall be construed as if such illegal, invalid or unenforceable provision had not been contained herein. The parties shall attempt to negotiate a provision replacing such provision and providing comparable benefits to each party, but in the event that such negotiations do not result in agreement within ninety (90) days, either party shall have the right to terminate this Agreement by ninety (90) days written notice to the other party.

8.6 No Strict Construction. The language used in this Agreement shall be deemed to be the language chosen by both parties hereto to express their mutual intent and no rule of strict construction against either party shall apply to any term or condition of this Agreement.

8.7 Relationship of Parties. Nothing contained in this Agreement shall be construed as creating a partnership, joint venture, agency or an association of any kind.



2           8.8 No Waiver. The failure of one party hereto to enforce at any time any of the  
4 provisions of this Agreement, or any rights in respect thereto, or to exercise any election herein  
6 provided, shall in no way be considered to be a waiver of such provision, rights or elections or in  
any way to affect the validity of this Agreement. Any waiver must be in writing.

8           8.9 Interpretation. The headings contained in this Agreement are for convenience only  
10 and shall not affect the interpretation of this Agreement. In this Agreement, the word "including"  
shall be deemed to be followed by "without limitation", the words "hereof" and "herein" and  
"hereunder" refer to this Agreement as a whole, and the singular includes the plural and vice versa.

12           8.10 Notices. Notices shall be given by first class mail, by Federal Express or other  
14 recognized courier requiring signature on receipt, or by telecopy confirmed by contemporaneous  
16 phone conversation with the recipient of the telecopy, and shall be addressed to the other party at  
the address set forth below (or at such address as a party may specify by notice to the other):

18                   If to Licensee:           Pioneer Hi-Bred International, Inc.

20                                   Attention: Vice President, T&TD  
22                                   700 Capitol Square 7300 NW 62<sup>nd</sup> Ave.  
24                                   400 Locust Street P.O. Box 1004  
                                  Des Moines, IA 50309-2349 Johnston, IA 50131-1004  
                                  Telephone: (515) 248-4800 (515) 270-3600  
                                  Telecopy: (515) 253-2478 (515) -253-2478

26                   if to Licensor:           Kimeragen, Inc.

28                                   Attention: \_\_\_\_\_  
30                                   300, Pheasant Run  
                                  Newtown, PA 18940  
                                  Telephone: (215) 504-4444  
32                                   Telecopy: (215) 504-4545

34           8.11 Governing Law. This Agreement shall be governed by and construed in accordance  
36 with the laws of Pennsylvania without giving effect to any choice of law or conflict of law provision  
or rule that would cause the application of the laws of any jurisdiction other than Pennsylvania.

38           8.12 Counterparts. This Agreement may be executed in one or more counterparts, each  
40 of which shall be deemed an original, but all of which together shall constitute one and the same  
instrument.

42           8.13 Dispute Resolution. The parties shall work together to remedy any difficulties which  
44 may arise in connection with this Agreement. All disputes arising out of this Agreement shall be  
referred to decision forthwith to a senior executive of each party who is, if possible, not involved in  
the dispute. If no agreement can be reached through this process within thirty (30) days of request  
46 by one party to the other to nominate a senior executive for dispute resolution, then either party  
hereto shall be entitled to refer such dispute to three arbitrators for arbitration, such arbitration to be

held in Chicago, Illinois on an expedited basis in accordance with the rules and regulations of the American Arbitration Association. One arbitrator shall be appointed by each party within thirty (30) days of a request for arbitration or receipt of notice thereof, with such arbitrators to appoint the third arbitrator within thirty (30) days of the appointment of the latter of the party arbitrators. The decision of the arbitrators shall be irrevocable and fully accepted by both parties.

\* \* \* \* \*

IN WITNESS WHEREOF, the parties have caused their duly authorized representative to execute this Agreement as of the Effective Date.

PIONEER HI-BRED INTERNATIONAL, INC.

By: \_\_\_\_\_

Name: \_\_\_\_\_

Title: \_\_\_\_\_

KIMERAGEN, INC.

By: \_\_\_\_\_

Name: \_\_\_\_\_

Title: \_\_\_\_\_

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**EXHIBIT A**

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**Patent Rights**

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**EXHIBIT B**

**Sublicense Provisions**

1. **Definitions.**

"Kimeragen" shall mean Kimeragen, Inc.

"Kimeragen Licensors" shall mean TJU and the Cornell Research Foundation, Inc. ("Cornell").

2. **Indemnities.** Licensee hereby waives any claim against Kimeragen and the Kimeragen Licensors and agrees to indemnify, defend, and hold harmless Kimeragen and the Kimeragen Licensors and their respective directors, officers, employees and agents, and in the case of the Kimeragen Licensors, their trustees, officers, agents and employees and those of any associated university, from all liabilities, demands, damages, expenses and losses (including without limitation for death, personal injury, illness or property damage, and including reasonable attorneys' fees) arising out of or in connection with this Agreement (collectively, the "Indemnified Losses"), including without limitation Indemnified Losses resulting from any exercise or use by Licensee or its transferees of Patent Rights, and any use, sale, or other disposition of Licensed Product by Licensee or its transferees and any claim that Licensee's use, sale, or other disposition of Licensed Product infringes or violates any patent or other intellectual property rights. The indemnification rights contained herein are in addition to all rights which Kimeragen and/or the Kimeragen Licensors may have at law or in equity. Licensee hereby agrees that the Kimeragen Licensors are entitled to enforce this Section directly against Licensee. Licensee shall cause its affiliates, subsidiaries, contractors and sub-contractors to waive claims against and indemnify the Kimeragen Licensors on the terms set forth above. Notwithstanding the foregoing, the indemnities provided by Licensee herein shall not apply to the extent the indemnified losses would be or are covered by the foregoing Licensors' warranties.

3. **Product Liability Insurance.** Licensee shall obtain and maintain commercial general liability insurance, including commercial liability, product liability and completed operations insurance coverage in minimum amount of five million dollars (\$5,000,000) per loss including coverage for contractual liability. Kimeragen and the Kimeragen Licensors and their respective officers, directors, trustees, members of governing boards and employees will be named insureds under all such insurance. Such insurance shall also provide that Licensors and the Kimeragen Licensors be given notice of any modification thereof and at least ten (10) days prior written notice of cancellation or termination and the reason therefor. A certificate of insurance evidencing such coverage will be provided to Kimeragen and the Kimeragen Licensors and, upon each annual anniversary of this Agreement, Licensee shall provide written confirmation issued by the insurer or an independent insurance agent

**EXHIBIT C**

**Royalty Calculations**

**[SUBJECT TO REVIEW]**

	ROYALTY	MAXIMUM ROYALTY*
Corn (80K unit)	\$0.250	\$0.50
Sorghum (50# unit)	\$0.134	\$0.268
Soybean (50# unit)	\$0.043	\$0.087
Sunflower (200K unit)	\$0.280	\$0.560
Canola (277750# unit)	<del>[\$0.000]</del> CND\$0.381	<del>[\$0.000]</del> CND\$0.762
Alfalfa (50# unit)	\$0.462	\$0.924

\* For more than one use of the Licensor Technology.

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confirming that insurance is maintained in accordance with the above requirements.  
At Licensee's sole determination, Licensee may elect to be self-insured, provided  
that such self-insurance is substantially equal to the coverage as described above.

4. Use of Names. Licensee shall not use the name of Kimeragen or any Kimeragen Licensor, or the names of any of their respective staff members, employees or students or any adaptation thereof in any advertising, promotional or sales literature to the extent such use might imply a relationship between the parties, or endorsement by Kimeragen or any Kimeragen Licensor of any act or thing or any product or method described in such material, without the prior written consent of Kimeragen and the Kimeragen Licensor where applicable, which consent shall not be unreasonably withheld.
5. Disclaimer of Warranties. LICENSOR HEREBY EXPRESSLY DISCLAIMS ANY AND ALL WARRANTIES AND REPRESENTATIONS, EXPRESS OR IMPLIED, ARISING BY LAW OR CUSTOM, WITH RESPECT TO THE LICENSOR TECHNOLOGY, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, PATENTABILITY OR NON-INFRINGEMENT. LICENSOR DOES NOT IN ANY WAY PROMISE THAT THE LICENSOR TECHNOLOGY SHALL PRODUCE ANY PARTICULAR RESULTS, PRODUCTS OR PROFITABILITY.
6. Quality Control. Licensee shall comply with all regulatory requirements relating to the Licensed Products and shall take all reasonable or required steps to ensure that the Licensed Products are safe and lawful.

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2 Draft 9/10/96 Kimeragen  
4 Draft 9/20/96 Pioneer

LICENSE AGREEMENT

"clear"

6  
8 THIS LICENSE AGREEMENT (the "Agreement") is made and entered into this \_\_\_\_ day of  
10 \_\_\_\_\_, 1996 (the "Effective Date") between Pioneer Hi-Bred International, Inc., an Iowa  
12 corporation of 700 Capitol Square, 400 Locust Street, Des Moines, IA 50309-2340 ("Licensee"),  
and Kimeragen, Inc., a Delaware corporation, of 300 Pheasant Run, Newtown, PA 18940  
("Licensor").

14 WHEREAS, Licensor is the exclusive licensee of Thomas Jefferson University ("TJU")  
16 under U.S. and foreign patent applications with respect to a chimeric vector for application in gene  
therapy developed by TJU and certain methods and processes using that chimeric vector; and

18 WHEREAS, Licensee wishes to obtain an exclusive, worldwide license to use the  
technology to make and sell products in the Licensee Field-of-Use (as defined below).

20 NOW THEREFORE, in consideration of the mutual promises and covenants set forth herein  
22 and for good and valuable consideration, the adequacy and sufficiency of which is hereby  
24 acknowledged, the parties hereby agree as follows:

26 ARTICLE 1-DEFINITIONS

28 1.1 "Affiliate" shall mean any company or entity related to Licensee or to a Subsidiary of  
30 Licensee by contract and through which Licensee or Subsidiary of Licensee produces and/or  
markets seed to end-user customers, but only for the period or periods during which such a  
contractual relationship exists.

32 1.2 "Chimera" shall mean the chimeric vector of RNA and DNA as claimed in the Patent  
34 Rights or otherwise the subject of non-patented Licensor Technology.

36 1.3 "Confidential Information" shall mean all information, in whatever form, which is  
disclosed by either party prior to or subsequent to the Effective Date of this Agreement.

38 1.4 "Intellectual Property" shall mean without limitation, all filed patents, patentable  
40 inventions, know-how, trade secrets, techniques and ideas and all technical material which further  
includes DNA and RNA sequences and genes, oligos and proteins, and associated methods and all  
42 applications therefor.

44 1.5 "Kimeragen Licensors" shall mean TJU and the Cornell Research Foundation, Inc.  
46 ("Cornell").

48 1.6 "Licensed Products" shall mean products containing genetic material that has been  
altered (in that generation or an earlier generation) by the use of the Licensor Technology.

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1.7 "Licensee Field-of-Use" shall mean Corn, Sunflower, Soybean, Canola/Rape, Alfalfa, Tobacco, *Arabidopsis* and Sorghum, provided that no Tobacco or *Arabidopsis* products or products produced by or from use of the Licensors Technology shall be sold by Licensee.

1.8 "Licensee Technology" shall mean all Intellectual Property conceived, controlled by, and/or developed by Licensee pertaining to the Chimera and associated methods required for its use.

1.9 "Licensor Field-of-Use" shall mean all uses outside the Licensee Field-of-Use.

1.10 "Licensor Technology" shall mean the Chimera, all Intellectual Property conceived, controlled by, and/or developed by Licensor covering the Chimera and other Intellectual Property of Licensor (including Rec2 gene (as defined below)) but use of any such Intellectual Property shall be limited to the extent required for the use of the Chimera in the Licensee Field-of-Use. Licensor Technology shall further include all improvements to Licensor Technology.

1.11 "Patent Rights" shall mean patents and patent applications and all foreign counterparts thereof in the Territory as listed in Exhibit A, including all future divisionals and continuations, continuations in part, additions, confirmations, renewals, extensions and reissues of patents and patent applications and their equivalents covering the Licensor Technology.

1.12 "Plants" shall mean multicellular rooted organisms containing chlorophyll and cellulose cell walls.

1.13 "Pharmaceutical Use" shall mean all uses related to pharmaceutical compounds that are or would be regulated by the FDA Centers of Biological Evaluation and Research (CBER), Drug Evaluation and Research (CDER), Devices and Radiologic Health (CDRH) and Veterinary Medicine (CVM) in any species, and all similar foreign agencies.

1.14 "Rec2" shall mean that gene as described in relation to *Ustilago* in U.S. patent application serial no. 08/373,134 ("*Ustilago* Rec2") and in relation to humans in patent disclosure Pennie & Edmonds docket no. 79910010 ("Human Rec2").

1.15 "Sublicensee" shall mean any company or entity which is sublicensed by Licensee pursuant to the terms of Section 2.2.

1.16 "Subsidiary" shall mean any company or entity at least 40% of whose voting stock is owned or directly or indirectly controlled by Licensee.

1.17 "Territory" shall mean the world.

## ARTICLE 2--LICENSE

2.1 Grant of License. Licensor hereby grants to Licensee, upon the terms and conditions set forth herein, a royalty-bearing, exclusive license under the Licensor Technology, including the Patent Rights, to use and make (pursuant to section 7.2) Chimera for research

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purposes and to the extent necessary to develop and commercialize Licensed Products in the Licensee Field-of-Use in the Territory.

(a) Licensee shall have the right to assign within the Licensee Field-of-Use or Territory (or part thereof) the license granted in this Section 2.1 to certain wholly-owned Subsidiaries; and

(b) Licensee shall have the right to subcontract to Licensee's Subsidiaries and Affiliates activities relating to the growing and marketing of Licensed Products within the Licensee Field-of-Use or Territory (or part thereof) for the purposes of conducting Licensee's ongoing business.

2.2 Notwithstanding any such assignment as per (a) above or subcontracting as per (b) above, Licensee shall be responsible for the activities, including all reporting and royalty obligations, of such Subsidiaries and Affiliates as if they were the Licensee.

2.3 Sublicensing. Licensee may grant sublicenses within the scope of the licenses granted in Section 2.1 to third parties, provided that:

(a) Licensor has the right pursuant to any applicable contract between Licensor and a third party (or third parties) to allow Licensee to sublicense the Intellectual Property right in question;

(b) Licensee shall cause all such Sublicensees to comply with the provisions of this Agreement in the same manner as Licensee is bound hereby and shall contain provisions protecting the Kimeragen Licensors as specified on Exhibit B;

(c) No Sublicensee shall have the further right to sublicense;

(d) Licensee shall be responsible for compliance by such Sublicensees with the terms of this Agreement; and

(e) Licensee shall give the Licensor prompt written notice of all sublicenses granted pursuant to this Section 2.2 as well as copy of the sublicense agreement.

2.4 Improvements. During the term provided in Section 6.2, the parties shall promptly disclose to each other in reasonable detail any newly developed Intellectual Property which is related to the Licensor Technology or Licensee Technology.

(a) Licensee hereby grants to Licensor an exclusive paid up worldwide license to the Licensee Technology, with the right to grant sublicenses (which may in turn include the right to grant further sublicenses), for all uses and applications in the Licensor Field-of-Use, provided that Licensor shall have no right to Licensee Technology for any uses and applications in Plants. The terms and conditions of

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such license shall be substantially like those contained in this present license recognizing that the parties to the new license are reversed.

**2.5 Limitations on Exclusivity.** Except as provided below, the licenses granted under Sections 2.1 and 2.3 shall be exclusive in the Territory in the respective field of use of each party.

(a) The licenses shall be non-exclusive to the extent required by government regulations; and

**2.6 Freedom to Use.** In the event that either party shall license third-party Intellectual Property to gain freedom-to-operate in that party's Field-of-Use and such Intellectual Property is also needed by the other party to gain freedom-to-operate in its Field-of-Use, subject to Section 3.6, the first party shall use all reasonable efforts to license such Intellectual Property so that other party's use of such Intellectual Property is covered. Neither party shall enter into any agreement that would preclude the other party from being able to also obtain a license.

**2.7 Protection of Technology.** Licensee shall not use the Licensor Technology for any purpose other than as provided in this Agreement. Licensee shall take no action in respect of the Licensor Technology which is inconsistent with the terms of the license granted under this Agreement.

**2.8 Acknowledgment of Rights.** Licensee acknowledges that Licensee's right to use the Licensor Technology arises only out of the licenses granted under this Agreement. All Licensed Products manufactured under issued patents shall bear a patent notice on the label as may be necessary or appropriate under the laws of the country in the Territory in which the Licensed Product is sold.

### **ARTICLE 3--PAYMENT**

**3.1 Payment.** In consideration of the rights granted herein, the Licensee shall pay to the Licensor:

(a) three million dollars (US\$3,000,000) upon the date of execution of this Agreement, provided that this Agreement shall not become effective until such payment is made. Such payment shall be for the use of the rights granted herein for a period extending from the Effective date to twenty (20) months after the Effective Date.

(b) two million dollars (US\$2,000,000) twenty (20) months after the Effective Date provided that Licensee shall not have issued a notice of termination pursuant to Section 6.3(a) effective on or before such date. Such payment shall be comprised of two portions: i) one million nine hundred thousand

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dollars (\$1,900,000) for patents and patent applications in the Patent Rights that are filed after June 8, 1995 or issued before twenty months after the Effective Date, and ii) one hundred thousand dollars (\$100,000) for patent applications and patents in the Patent Rights but not included in i) above.

(c) a royalty per unit of Licensed Product as specified on Exhibit C.

(d) a portion of sublicensing fees and/or royalties as provided in Section 3.3.

3.2 Royalty Conditions. Royalties shall:

(a) be payable on the sale by Licensee (including Licensee's Affiliates, Subsidiaries or sublicensees) of Licensed Products to end-use customers, but only when such Licensed Products' sale, development or production is, at the time of sale covered by an Issued Patent Right in the country where Licensed Product is sold, developed or produced;

(b) accrue upon the sale of Licensed Product (including Affiliates and Subsidiaries) as determined by U.S. GAAP;

(c) be due and payable in U.S. dollars annually within sixty (60) days after the close of Licensee's fiscal year (August 31);

(d) not be due and payable on i) Licensed Products used for Licensee's internal purposes (test beds) but shall be due on samples provided to end-use customers, or ii) sales of Licensed Product to or among Licensee and Licensee's Affiliates, Subsidiaries or Sublicensees;

(e) be prorated based on the package sizes shown on Exhibit C for package sizes not shown on Exhibit C;

(f) be due and payable on Licensed Products used by Licensee for the production of commercial grain at the same royalty amount as if the Licensed Products were sold.

3.3 Sublicenses. Licensor shall be entitled to share in all sublicensing fees on a 50:50 basis. In the event that Licensee barter or trades the rights as granted in Section 2.1 to a third party for other technology, or otherwise shall receive non-cash consideration, no fees shall be due to Licensor. Licensee shall be obligated to pay royalties to Licensor on sales by such third party as if such sales were sales by Licensee at the rate provided on Exhibit C. Licensee shall be responsible for monitoring and reporting on the sales of its Sublicensees and shall be responsible for all royalties due to Licensor.

3.4 Reports. Licensee shall provide royalty reports to Licensor within sixty (60) days after the close of Licensee's fiscal year. Such reports shall contain information about Licensee's sales as well as sales by and revenue received from Sublicensees reasonably necessary to verify royalties due, including:

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- (a) A listing of all Licensed Products qualifying for royalties sold by Licensee (including Subsidiaries and Affiliates) and Sublicensees, sorted by individual product, crops and country;
- (b) Royalty calculation; and
- (c) Royalties due and payable.

**3.5 Audit.** The Licensee shall keep full and accurate records at its principal place of business in the United States of all Licensed Products grown, distributed, or sold by Licensee (including Subsidiaries and Affiliates) and its Sublicensees during the term of this Agreement. Such records shall be open, at reasonable times, for three (3) years following the end of the fiscal year to which they pertain, to an inspection by a mutually agreed upon Certified Public Accounting Firm, at Licensors expense, for the purposes of verifying Licensee's royalty payments under this Agreement. If a discrepancy of greater than five percent (5%) is found in any payment due hereunder, Licensee shall reimburse Licensors for the cost of such inspection and promptly pay such overdue amount together with interest at the rate specified in Section 3.7.

**3.6 Third Party Royalties.** In any case where use of the Licensors Technology licensed to Licensee is in the future subject to a royalty (whether lump-sum or payable by reference to sales) to a third party other than Kimbergen Licensors with respect to currently licensed Licensors Technology, then in the event that Licensee determines to use such Licensors Technology, Licensee shall, in addition to the royalty specified in Section 3.1, be responsible for payment to Licensors of a further amount equal to the royalty payable to the third party attributable to sales by Licensee.

**3.7 Late Payments.** All payments to be made by the Licensee to the Licensors hereunder shall bear interest at the prime or equivalent rate as quoted by Citibank N.A., New York, New York, on the day the payment is overdue plus two percent (2%) per annum until paid.

#### **ARTICLE 4--PATENTS**

**4.1 Prosecution.** Licensors shall use reasonable efforts to prosecute the Patent Rights to the extent within the control of Licensors. In the event that Licensors shall elect not to continue prosecution such that the Chimera would not be subject to patent protection, then Licensee shall have the right to assume such prosecution at its expense, but any royalty obligation relating solely thereto shall cease.] [Chimera patent about to issue.]

**4.2 Infringement Actions.** Licensee shall notify Licensors promptly of any action, claim or threat of patent infringement suit, either oral or written, or the commencement of any such patent infringement suit against Licensee relating to the Licensors Technology or of any infringement by a third party of the Patent Rights within the Licensee Field-of-Use. The parties shall cooperate in the development and execution of a strategy to defend against any such action against Licensee or to prevent any such infringement by a third party.

#### **ARTICLE 5--WARRANTIES & INDEMNITIES**

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5.1 Warranties.

- (a) Each party represents and warrants to the other that it has full right, power and authority to enter into this Agreement and that the terms of this Agreement do not conflict with any other contractual obligations it has.
- (b) Licensor represents and warrants that, as of the execution date, it knows of no Intellectual Property that would prevent Licensee from practicing the Licensor Technology.
- (c) Licensor represents and warrants that it has the freedom to enter into this Agreement and the right to provide the rights and license contemplated in Section 2.1.
- (d) Licensor represents and warrants that, as of the Effective Date, Licensor has disclosed to Licensee all known, and knows of no other, royalty obligations pursuant to Section 3.6;
- (e) No Other Warranties. Licensor makes no other warranty or representation with respect to the Licensor Technology, nor is Licensor in any way responsible for the utility of any Licensor Technology. LICENSOR HEREBY EXPRESSLY DISCLAIMS ANY AND ALL WARRANTIES AND REPRESENTATIONS, EXPRESS OR IMPLIED, ARISING BY LAW OR CUSTOM, WITH RESPECT TO THE LICENSOR TECHNOLOGY, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, PATENTABILITY OR NON-INFRINGEMENT. LICENSOR DOES NOT IN ANY WAY PROMISE THAT THE LICENSOR TECHNOLOGY SHALL PRODUCE ANY PARTICULAR RESULTS, PRODUCTS OR PROFITABILITY.

5.2 Indemnities. Licensee hereby waives any claim against Licensor and the Kimeragen Licensors and agrees to indemnify, defend, and hold harmless Licensor and the Kimeragen Licensors and their respective directors, officers, employees and agents, and in the case of the Kimeragen Licensors, their trustees, officers, agents and employees and those of any associated university, from all liabilities, demands, damages, expenses and losses (including without limitation for death, personal injury, illness or property damage, and including reasonable attorneys' fees) arising out of or in connection with this Agreement (collectively, the "Indemnified Losses"), including without limitation Indemnified Losses resulting from any exercise or use by Licensee or its transferees of Patent Rights, and any use, sale, or other disposition of Licensed Product by Licensee or its transferees and any claim that Licensee's use, sale, or other disposition of Licensed Product infringes or violates any patent or other intellectual property rights. The indemnification rights contained herein are in addition to all rights which Licensor and/or the Kimeragen Licensors may have at law or in equity. Licensee hereby agrees that the Kimeragen Licensors are entitled to enforce this Section 5.2 directly against Licensee. Licensee shall cause its Affiliates and Subsidiaries, contractors and sub-contractors to waive claims against and indemnify the Kimeragen Licensors on the terms set forth above. Notwithstanding the foregoing, the indemnities provided by

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Licensee herein shall not apply to the extent the indemnified losses would be or are covered by the foregoing Licensor's warranties.

#### ARTICLE 6--TERM

6.1 Term Of Agreement. This Agreement shall become effective as of its signature by both parties and payment by Licensee of the sum specified in Section 3.1(a). All obligations hereunder shall expire at the last to expire of any Patent Right having a valid claim which Licensee would infringe by its sale of Licensed Products but for this Agreement.

6.2 Term of Technology Transfer. The transfer of improvements as foreseen in Section 2.3 shall cease on the eight (8<sup>th</sup>) anniversary of the Effective Date.

#### 6.3 Early Termination.

(a) Licensee shall have the right to terminate this Agreement by at least sixty (60) days written notice to Licensor.

(b) Either party may terminate this Agreement with immediate effect by written notice to the other party, if the other party goes into bankruptcy or insolvency, or the other party commits a material breach of any material obligation under this Agreement and fails to remedy such breach within sixty (60) days after notice of such breach.

6.4 Restriction of Licensee Field-of-use. Licensor shall be entitled to modify Licensee Field-of-Use on a crop-by-crop basis upon thirty (30) days written notice to Licensee if Licensee ceases, for twelve (12) consecutive months, after the year 2003, to develop or sell Licensed Products within such species.

6.5 Effects of Termination. Termination of this Agreement shall not affect the continued enforceability of Sections \_\_\_\_\_

#### ARTICLE 7--OTHER AGREEMENTS

7.1 Supply of Chimera. In order to ensure the quality of the Chimera, Licensor shall supply or arrange for supply of Chimera to Licensee at an initial price of one thousand dollars (US\$1,000) per micromole unpurified and one thousand two hundred dollars (US\$1,200) per micromole (HPLC purified). Such prices shall not be increased except to the extent of increases in Licensor's costs. Licensor shall make a reasonable effort to supply the agreed upon quantity of Chimera within fifteen (15) days of receipt of the order. Payments shall be made net thirty (30) days from delivery. Licensee shall be required to provide forecasts of its requirements for Chimera.

7.2 Obligation to Purchase of Chimera. Upon the second anniversary of the Effective date, Licensee may make internally or have made or buy from another vendor Chimera for its own internal use. Licensee shall not be required to purchase Chimera in any country where such a requirement would be illegal or render unenforceable Licensor's Patent Rights.

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2           **7.3 Committee.** A committee comprising an equal number of representatives of each  
3 party shall be formed (the "Committee") and shall meet not less than once each calendar year to  
4 discuss research, progress toward commercialization and commercialization of Licensed Products.  
5 Licensee shall provide a research plan with benchmarks for development and commercialization of  
6 Licensed Products in each crop within the Licensee Field-of-Use. Prior to each such meeting,  
7 Licensee shall provide Licensor with a written report setting out research conducted measured  
8 against benchmarks, and summarizing progress toward commercialization and, when Licensed  
9 Products are available for commercial sale, a summary of marketing and sales figures and trends.  
10 The Committee shall also discuss joint opportunities for licensing of technology for Plants outside  
11 Licensee Field-of-Use.

12           **7.4 Diligence.** Licensee shall use reasonable commercial efforts to develop and  
13 commercialize the Licensed Products.

14           **7.5 Research.** Licensee shall be primarily responsible for development and execution of  
15 research in Licensee Field-of-Use. Licensor shall provide reasonable access to its consultants and  
16 employees to assist Licensee with the technology at a fee of \$2,000 per full day of services plus  
17 reasonable expenses. The parties may agree to undertake joint research, in which case the parties  
18 shall negotiate in good faith to agree to the terms upon which such research shall be conducted  
19 and a research plan. [During the first [three years after the Effective Date,] Licensor shall  
20 provide Licensee with up to five (5) days per year of Dr. Kmiec's consulting time per year at  
21 two thousand dollars (US\$2,000) per day and up to twenty (20) days per year of Dr. Kumar's  
22 (or other Licensor's scientists') consulting time at no cost to Licensee, and up to twenty (20)  
23 hours per year at two thousand dollars (US\$2,000) per day.] [Subject to review]

24           **7.6 Trademarks and Use of Names.** If Licensee develops a Licensed Product for  
25 commercialization, the Committee shall consider in good faith whether use of Licensor's  
26 trademarks or a description of Chimera technology in marketing brochures, literature and labeling  
27 for any Licensed Product is appropriate and desirable, and the appropriate payment and other  
28 terms for such trademark use. Neither party shall use the name of the other, nor shall Licensee  
29 use the name of any Kimeragen Licensor, or the names of any of their respective staff members,  
30 employees or students or any adaptation thereof in any advertising, promotional or sales literature  
31 to the extent such use might imply a relationship between the parties, or endorsement by either  
32 party or any Kimeragen Licensor of any act or thing or any product or method described in such  
33 material, without the prior written consent of the other party and the Kimeragen Licensor where  
34 applicable, which consent shall not be unreasonably withheld.

35           **7.7 Confidentiality.** Each party shall:

- 36           (a) maintain the Confidential Information of the other party in confidence and  
37 refrain from disclosing any part of such Confidential Information to any  
38 person or entity other than to its employees, consultants and sublicensees  
39 whose duties or rights justify the need to know such Confidential Information;
- 40           (b) not to make any use of the Confidential Information other than for the  
41 purpose of carrying out duties and obligations under this Agreement;

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- (c) to take all reasonable steps to protect the Confidential Information against disclosure, misuse, loss and theft, which steps include the execution by all such persons of written agreements containing obligations of confidentiality, restricted disclosure and limited use relative thereto consistent with this Section 7.6 prior to disclosure of Confidential Information to them; and
- (d) in the event that a third party wishes to evaluate Confidential Information in connection with a proposed business transaction with a party, disclose only as much of the Confidential Information to that third party as is necessary to conduct such evaluation, provided that prior to disclosure such third party executes a written agreement prohibiting use of the Confidential Information for any reason other than evaluation of such transactions and containing obligations of confidentiality consistent with this Section 7.6.

The provisions of this Section 7.6 shall not apply to any part of the Confidential Information disclosed by one party to the other (a) which is agreed in writing by the disclosing party to be excluded; or (b) which the receiving party can show was known to or developed by it prior to Confidential Information first being received by it from, or disclosed to it by, the disclosing party; or (c) which is public knowledge, or becomes public knowledge in the future, other than through acts or omissions of the receiving party in breach of this Agreement; or (d) which is lawfully obtained by receiving party from sources independent of the disclosing party who have a lawful right to possess and disclose such Information; or (e) which it is necessary for the receiving party to disclose in order to comply with any applicable law or if required to do so by order of any court or any other judicial or administrative body, provided that prior to making such disclosure the receiving party gives the disclosing party notice of the requirement of disclosure and the information to be disclosed.

**7.8 Press Releases.** Prior to any press release concerning this Agreement, both parties shall agree on the content and timing, such consent not to be unreasonably withheld.

**7.9 Regulatory Approvals.** The parties shall provide to one another (at no cost) all materials, data and information in their possession needed to seek and obtain regulatory approvals necessary for the use and sale of products in their respective Fields-of-Use which use the Licensed Technology. Neither party shall use any regulatory information and/or packages developed by the other for the benefit of a third party. Licensee shall comply with all regulatory requirements relating to the Licensed Products and shall take all reasonable or required steps to ensure that the Licensed Products are safe and lawful.

**7.10 Product Liability Insurance.** Licensee shall obtain and maintain commercial general liability insurance, including commercial liability, product liability and completed operations insurance coverage in a minimum amount of five million dollars (\$5,000,000) per loss including coverage for contractual liability. Licensor and the Kimeragen Licensors and their respective officers, directors, trustees, members of governing boards and employees will be named insureds under all such insurance. Such insurance shall also provide that Licensor and the Kimeragen Licensors be given notice of any modification thereof and at least ten (10) days prior written notice of cancellation or termination and the reason therefor. A certificate of insurance evidencing such



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coverage will be provided to Licensor and the Kimeragen Licensors and, upon each annual anniversary of this Agreement, Licensee shall provide written confirmation issued by the insurer or an independent insurance agent confirming that insurance is maintained in accordance with the above requirements. At Licensee's sole determination, Licensee may elect to be self-insured in accordance with reasonable business practices.

7.11 Upon request from Licensee, Licensor shall diligently undertake to have this licensing agreement registered by the competent authorities of the countries of the Territory in order to safeguard Licensee's ability to join Licensor in any Intellectual Property rights enforcement action brought by Licensor against a third party and allow Licensee to claim damages resulting from the violation of the Intellectual Property rights licensed to him.

#### **ARTICLE 8--MISCELLANEOUS PROVISIONS**

8.1 **Force Majeure.** Neither party shall be liable for failure to perform its obligations hereunder for so long as that failure may be the result of an event beyond its reasonable control (a "force majeure" event), provided that such party uses all reasonable efforts to comply with the terms of this Agreement to the extent that it is able to do so.

8.2 **Entire Agreement.** This Agreement, together with all Exhibits attached hereto, constitutes the entire Agreement between the parties with respect to the present subject matter, all prior negotiations, agreements and understandings being expressly canceled hereby.

8.3 **Amendment.** This Agreement may be amended only by a written agreement embodying the full terms of the amendment signed by authorized representatives of both parties.

8.4 **Assignment.** Neither party may assign their rights or obligations under this Agreement without prior written approval from the other party, except i) as set forth in Article 2 and ii) as part of the sale or transfer of substantially all the business to which this Agreement pertains, provided that such purchaser expressly agrees to assume that party's rights and obligations under this Agreement.

8.5 **Severability.** Should any provision of this Agreement be illegal, invalid or unenforceable under applicable law, the remaining provisions of this Agreement shall be construed as if such illegal, invalid or unenforceable provision had not been contained herein. The parties shall attempt to negotiate a provision replacing such provision and providing comparable benefits to each party, but in the event that such negotiations do not result in agreement within ninety (90) days, either party shall have the right to terminate this Agreement by ninety (90) days written notice to the other party.

8.6 **No Strict Construction.** The language used in this Agreement shall be deemed to be the language chosen by both parties hereto to express their mutual intent and no rule of strict construction against either party shall apply to any term or condition of this Agreement.

8.7 **Relationship of Parties** Nothing contained in this Agreement shall be construed as creating a partnership, joint venture, agency or an association of any kind.

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2           8.8 No Waiver. The failure of one party hereto to enforce at any time any of the  
4 provisions of this Agreement, or any rights in respect thereto, or to exercise any election herein  
6 provided, shall in no way be considered to be a waiver of such provision, rights or elections or in  
any way to affect the validity of this Agreement. Any waiver must be in writing.

8           8.9 Interpretation. The headings contained in this Agreement are for convenience only  
10 and shall not affect the interpretation of this Agreement. In this Agreement, the word "including"  
shall be deemed to be followed by "without limitation", the words "hereof" and "herein" and  
"hereunder" refer to this Agreement as a whole, and the singular includes the plural and vice versa.

12           8.10 Notices. Notices shall be given by first class mail, by Federal Express or other  
14 recognized courier requiring signature on receipt, or by telecopy confirmed by contemporaneous  
phone conversation with the recipient of the telecopy, and shall be addressed to the other party at  
the address set forth below (or at such address as a party may specify by notice to the other):

16                   If to Licensee:           Pioneer Hi-Bred International, Inc.

18                                   Attention: Vice President, T&TD \_\_\_\_\_  
20                                   7300 NW 62<sup>nd</sup> Ave.  
22                                   P.O. Box 1004  
Johnston, IA 50131-1004  
24                                   Telephone: (515) 270-3600  
Telecopy:   (515) -253-2478

26                   if to Licensor:           Kimragen, Inc.

28                                   Attention: \_\_\_\_\_  
30                                   300, Pheasant Run  
Newtown, PA 18940  
32                                   Telephone: (215) 504-4444  
Telecopy:   (215) 504-4545

34           8.11 Governing Law. This Agreement shall be governed by and construed in accordance  
36 with the laws of Pennsylvania without giving effect to any choice of law or conflict of law provision  
or rule that would cause the application of the laws of any jurisdiction other than Pennsylvania.

38           8.12 Counterparts. This Agreement may be executed in one or more counterparts, each  
40 of which shall be deemed an original, but all of which together shall constitute one and the same  
instrument.

42           8.13 Dispute Resolution. The parties shall work together to remedy any difficulties which  
44 may arise in connection with this Agreement. All disputes arising out of this Agreement shall be  
referred to decision forthwith to a senior executive of each party who is, if possible, not involved in  
the dispute. If no agreement can be reached through this process within thirty (30) days of request  
46 by one party to the other to nominate a senior executive for dispute resolution, then either party  
hereto shall be entitled to refer such dispute to three arbitrators for arbitration, such arbitration to be

Kimeragen, Inc./Pioneer Hi-Bred International, Inc.

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held in Chicago, Illinois on an expedited basis in accordance with the rules and regulations of the American Arbitration Association. One arbitrator shall be appointed by each party within thirty (30) days of a request for arbitration or receipt of notice thereof, with such arbitrators to appoint the third arbitrator within thirty (30) days of the appointment of the latter of the party arbitrators. The decision of the arbitrators shall be irrevocable and fully accepted by both parties.

\* \* \* \* \*

IN WITNESS WHEREOF, the parties have caused their duly authorized representative to execute this Agreement as of the Effective Date.

PIONEER HI-BRED INTERNATIONAL, INC.

By: \_\_\_\_\_

Name: \_\_\_\_\_

Title: \_\_\_\_\_

KIMERAGEN, INC.

By: \_\_\_\_\_

Name: \_\_\_\_\_

Title: \_\_\_\_\_

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**EXHIBIT A**

4

**Patent Rights**

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**EXHIBIT B**

**Sublicense Provisions**

1. **Definitions.**

"Kimeragen" shall mean Kimeragen, Inc.

"Kimeragen Licensors" shall mean TJU and the Cornell Research Foundation, Inc. ("Cornell").

2. **Indemnities.** Licensee hereby waives any claim against Kimeragen and the Kimeragen Licensors and agrees to indemnify, defend, and hold harmless Kimeragen and the Kimeragen Licensors and their respective directors, officers, employees and agents, and in the case of the Kimeragen Licensors, their trustees, officers, agents and employees and those of any associated university, from all liabilities, demands, damages, expenses and losses (including without limitation for death, personal injury, illness or property damage, and including reasonable attorneys' fees) arising out of or in connection with this Agreement (collectively, the "Indemnified Losses"), including without limitation Indemnified Losses resulting from any exercise or use by Licensee or its transferees of Patent Rights, and any use, sale, or other disposition of Licensed Product by Licensee or its transferees and any claim that Licensee's use, sale, or other disposition of Licensed Product infringes or violates any patent or other intellectual property rights. The indemnification rights contained herein are in addition to all rights which Kimeragen and/or the Kimeragen Licensors may have at law or in equity. Licensee hereby agrees that the Kimeragen Licensors are entitled to enforce this Section directly against Licensee. Licensee shall cause its affiliates, subsidiaries, contractors and sub-contractors to waive claims against and indemnify the Kimeragen Licensors on the terms set forth above. Notwithstanding the foregoing, the indemnities provided by Licensee herein shall not apply to the extent the indemnified losses would be or are covered by the foregoing Licensor's warranties.

3. **Product Liability Insurance.** Licensee shall obtain and maintain commercial general liability insurance, including commercial liability, product liability and completed operations insurance coverage in minimum amount of five million dollars (\$5,000,000) per loss including coverage for contractual liability. Kimeragen and the Kimeragen Licensors and their respective officers, directors, trustees, members of governing boards and employees will be named insureds under all such insurance. Such insurance shall also provide that Licensor and the Kimeragen Licensors be given notice of any modification thereof and at least ten (10) days prior written notice of cancellation or termination and the reason therefor. A certificate of insurance evidencing such coverage will be provided to Kimeragen and the Kimeragen Licensors and, upon each annual anniversary of this Agreement, Licensee shall provide written confirmation issued by the insurer or an independent insurance agent

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confirming that insurance is maintained in accordance with the above requirements.  
At Licensee's sole determination, Licensee may elect to be self-insured, provided that such self-insurance is substantially equal to the coverage as described above.

4. Use of Names. Licensee shall not use the name of Kimeragen or any Kimeragen Licensor, or the names of any of their respective staff members, employees or students or any adaptation thereof in any advertising, promotional or sales literature to the extent such use might imply a relationship between the parties, or endorsement by Kimeragen or any Kimeragen Licensor of any act or thing or any product or method described in such material, without the prior written consent of Kimeragen and the Kimeragen Licensor where applicable, which consent shall not be unreasonably withheld.
5. Disclaimer of Warranties. LICENSOR HEREBY EXPRESSLY DISCLAIMS ANY AND ALL WARRANTIES AND REPRESENTATIONS, EXPRESS OR IMPLIED, ARISING BY LAW OR CUSTOM, WITH RESPECT TO THE LICENSOR TECHNOLOGY, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, PATENTABILITY OR NON-INFRINGEMENT. LICENSOR DOES NOT IN ANY WAY PROMISE THAT THE LICENSOR TECHNOLOGY SHALL PRODUCE ANY PARTICULAR RESULTS, PRODUCTS OR PROFITABILITY.
6. Quality Control. Licensee shall comply with all regulatory requirements relating to the Licensed Products and shall take all reasonable or required steps to ensure that the Licensed Products are safe and lawful.

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**EXHIBIT C**

**Royalty Calculations**

**[SUBJECT TO REVIEW]**

	ROYALTY	MAXIMUM ROYALTY*
Corn (80K unit)	\$0.250	\$0.50
Sorghum (50# unit)	\$0.134	\$0.268
Soybean (50# unit)	\$0.043	\$0.087
Sunflower (200K unit)	\$0.280	\$0.560
Canola (50# unit)	CND\$0.381	CND\$0.762
Alfalfa (50# unit)	\$0.462	\$0.924

\* For more than one use of the Licensor Technology

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PIONEER HI-BRED INTERNATIONAL, INC.  
RESEARCH AND PRODUCT DEVELOPMENT

RESEARCH CENTER  
7300 N.W. 62ND AVENUE • P.O. BOX 1004  
JOHNSTON, IOWA 50131-1004  
PHONE: (515) 270-3600  
TELEFAX: (515) 270-4312

September 20, 1996

Mr. Bo Allen  
Senior Vice President  
300 Pheasant Run  
Newtown, PA 18940

Dear Bo,

First, let me reiterate that Pioneer continues to be very interested in Kimeragen's technology and pleased with our relationship with you and your colleagues. Like you, we are anxious to conclude this transaction as soon as practicable. Before proceeding, I would like to commend Stephen for an excellent job in capturing the parties' intentions.

I have attached our response to your draft of September 10<sup>th</sup>. At first glance, you will see that we have made a considerable number of changes. However, upon further review, I believe you will find that, with few exceptions, our changes were made to add clarity and definitiveness.

My greatest challenge at Pioneer is to be sure that one, our agreements can be readily understood by lay people, thus helping guarantee that Pioneer will meet all of its obligations, and two, the wording of the agreement reflects Pioneer's business structure in such a way as to not be limiting. It is in this spirit that I have made most of the changes.

In the interest of time, I will not explain the rationale for each and every change in writing, but I will try to cover some of the more substantial changes.

Second Whereas: Changed to more accurately reflect the grant and the relationship between our rights under the grant and the right to sell Licensed Products.

Article 1.7: Redrafted, but no change in scope or intent (at least no change intended).

Article 1.10: Improvements are specifically included in the grant pursuant to Section 2.3. For clarity, I have incorporated this concept in the definition.

Articles 1.14 and 1.16: After taking a close look at these definitions, it appeared that they do not materially help define the field, grant or obligation to pay royalties, so they were deleted.

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Article 2.1: The grant has been redrafted, primarily because 1.14 and 1.16 were deleted. However, we have added the right to "make", principally due to Article 7.1 (now new Article 7.2). We have also introduced a new 2.1(a) - this change does not impact our obligation to track and pay royalties or the total amount due to Kimeragen (see Section 2.2), rather it merely reflects the idiosyncrasies of our business structure.

Article (old 2.3) 2.4: Redrafted to clarify the term "improvement" and to somewhat define the terms and conditions of the grant-back.

Article (old 2.4) 2.5: Section (a) is an issue for use - philosophically it may make sense, but in practice its problematic because it suggests that our license may revert to non-exclusive for events completely out of our control. Section (b) is a problem because our license is exclusive for both commercial as well as research purposes in our field-of-use, which this provision contradicts.

Article (old 2.6) 2.7: Deleted to avoid redefining the rights granted under the license.

Article 2.8: We discussed this briefly over the phone - additional discussion is needed.

Article 3.1: We have not changed the timing or magnitude of the payments. Rather, we have further defined them, primarily in response to suggestions made by our tax consultants.

Article 3.2: Part of this Article has been extensively redrafted, primarily to clarify what event triggers a royalty obligation. From experience, this section will be the most widely read (and misunderstood), so it must be absolutely clear and concise. I do not believe our changes have, in any way, changed Pioneer's obligation to pay royalties on all Licensed Products which are covered by an issued patent.

✓ Article 3.3: The changes made here do represent a deviation from our previous agreement. Conceptually, I think we agree that Kimeragen's goal is to collect \$.25 for every unit of corn sold around the world, but we also agreed not to leave this issue to the committee. However, given that small changes in the language yielded the desired result, I opted to make the changes and propose that we deal with this issue now instead of at some later date.

Article 3.5: A sensitive issue for Pioneer.

Article 5.1: This change relates back to Article 3.6. While we do not like the concept of additional royalties as suggested in Article 3.6, we understand the reality of the situation.

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Bo Allen  
September 20, 1996

3

We would, however, ask that Kimeragen disclose these obligations as part of their reps and warranties.

Article 5.2: We have added language to the end of 5.2 which excludes the warranties described in Article 5.1 from the indemnification.

Article 6.2: We prefer eight years to five, but I am not sure this is a significant issue for Pioneer.

Article (new) 7.2: To fully exploit the value of the technology (and hence, maximize the royalty payment to Kimeragen), we would like the flexibility to make or have made (to the extent that this is possible given Kimeragen's patent estate) our own chimera. Given that Kimeragen has agreed to provide chimera at cost and, therefore, not a source of profit for Kimeragen, we are unclear why we are restricted from making our own.

Article (old 7.2) 7.3: Quarterly meetings are too frequent - given the nature of our product development cycle, yearly meetings should be sufficient.

Article (old 7.4) 7.5: More discussion is needed here. However, I think we agree that Kimeragen will make a concerted effort to transfer the technology to Pioneer without significant additional cost to Pioneer.

Article (old 7.9) 7.10: Pioneer is self-insured at a level that should be adequate.

Article 7.11: Our European counsel has asked that this provision be added. While the licensor typically does this and, therefore, we seldom include this language, our European counsel has recommended that it be included.

Article 8.4: This change was necessary given the changes proposed in Article 2.

That about does it. Please look over our proposed changes and let me know if you have any questions. Also, please let me know if I need to send a disk or electronic copy to Stephen. Thank you for your cooperation.

Sincerely,



Peter A. Fuller  
Director, Technology Identification & Access

Attachments: Redline and clean copy of Kimeragen/Pioneer License.

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**KIMERAGEN, Inc.**  
Molecular Pharmaceuticals

*Bo Allen  
Senior Vice President  
Business Development & Marketing*

November 20, 1996

Peter A. Fuller  
Director  
Technology Identification & Access  
Pioneer Hi-Bred International, Inc.  
Research Center  
7300 N.W. 62<sup>nd</sup> Avenue  
P.O. Box 1004  
Johnston, IA 50131-1004

Dear Peter,

Unfortunately, there were delays by our attorneys in preparing this preliminary draft of a non-exclusive world wide license for Kimeragen's technology. These delays made it impossible to get it to you in a timely fashion. Please accept my apologies.

Warmest regards,

Carroll "Bo" Allen  
Senior Vice President  
Marketing and Business Development

/ies

enclosure

cc: Gerald Messerschmidt

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**KIMERAGEN, Inc.**  
Molecular Pharmaceuticals

*Bo Allen  
Senior Vice President  
Business Development & Marketing*

November 20, 1996

Tony Cavalieri  
Pioneer Hi-Bred International, Inc.  
Research Center  
7300 N.W. 62<sup>nd</sup> Avenue  
P.O. Box 1004  
Johnston, IA 50131-1004

Dear Tony,

Unfortunately, there were delays by our attorneys in preparing this preliminary draft of a non-exclusive world wide license for Kimeragen's technology. These delays made it impossible to get it to you in a timely fashion. Please accept my apologies.

Warmest regards,

Carroll "Bo" Allen  
Senior Vice President  
Marketing and Business Development

/ies

enclosure

cc: Gerald Messerschmidt

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**KIMERAGEN, Inc.**  
Molecular Pharmaceuticals

*Bo Allen*  
*Senior Vice President*  
*Business Development & Marketing*

November 21, 1996

Peter A. Fuller  
Director  
Technology Identification & Access  
Pioneer Hi-Bred International, Inc.  
Research Center  
7300 N.W. 62<sup>nd</sup> Avenue  
P.O. Box 1004  
Johnston, IA 50131-1004

Dear Peter,

Unfortunately, there were delays by our attorneys in preparing this preliminary draft of a non-exclusive world wide license for Kimeragen's technology. These delays made it impossible to get it to you in a timely fashion. Please accept my apologies.

Warmest regards,

Carroll "Bo" Allen  
Senior Vice President  
Marketing and Business Development

/ies

enclosure

cc: Gerald Messerschmidt  
Leonard Shaykin

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Pioneer Hi-Bred International, Inc.  
7300 NW 62<sup>nd</sup> Avenue  
Johnston, IA. 50131-1004  
PH: 515/270-3300

## **FAX**

**TO: Iris  
Kimeragin**

**FROM: Nancy Christianson  
Pioneer Hi-Bred Intn'l., Inc.**

**Fax #: 215/504-4545**

**PH: 515/270-3956  
Fax: 515/253-2478**

**Iris:**

Room reservations have been made for Beau Allen and Gerald Messerschmidt at the University Park Holiday Inn—1800-50<sup>th</sup> Street, West Des Moines, IA. 515/223-1800 and guaranteed for late arrival on the evening of December 8. The confirmation number for Gerald-#66750116, Beau-#66751483.

Pickup at the Des Moines International Airport has been arranged with LIMO SERVICE which is owned and operated by Marsha Millard—Mobile Ph: 280-0124. She will pickup Beau and Gerald outside the baggage claim area in the transportation lane ahead of the taxi's. If for some reason the vehicle is not there, please call the mobile number listed above. Marsha is very punctual, so I doubt you will have a problem.

I have also made arrangements for Marsha's Limo Service to pickup Beau and Gerald at 8:00am at the University Park and transport them to the Pioneer Campus for an 8:30 meeting with Tony Cavalieri and Peter Fuller.

If you have any questions or need additional information, please call me at the above phone number.

Thanks,

Nancy

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1 **LICENSE AGREEMENT**

3 THIS LICENSE AGREEMENT (the "Agreement") is made and entered into this 10<sup>th</sup> day  
5 of March, 1997 (the "Effective Date") between Pioneer Hi-Bred International, Inc., an Iowa  
7 Company, of 400 Locust Street, Suite 700, Des Moines, IA 50309-2340 ("Licensee"), and  
Kimeragen, Inc., a Delaware corporation, of 300 Pheasant Run, Newtown, PA 18940 ("Licensor").

9 WHEREAS, Licensor represents that it is the exclusive licensee of Thomas Jefferson  
11 University ("TJU") under U.S. patent and certain U.S. and foreign patent applications with respect  
to a chimeric vector for application in gene therapy developed by TJU and certain methods and  
13 processes using that chimeric vector; and

15 WHEREAS, Licensee wishes to obtain a non-exclusive, worldwide license under such  
technology to conduct research with respect to Plants (as defined below) and to use, sell and license  
17 Licensed Products (as defined below).

19 NOW THEREFORE, in consideration of the mutual promises and covenants set forth herein  
and for good and valuable consideration, the adequacy and sufficiency of which is hereby  
acknowledged, the parties hereby agree as follows:

21 **ARTICLE 1 - DEFINITIONS**

23 1.1 "Affiliate" shall mean any person, which directly or indirectly controls, or is under  
25 common control with, or is controlled by, Licensee. "Control" shall mean the power to direct or  
cause the direction of the management and policies of a person, whether through the ownership of  
27 voting securities by contract or otherwise. "Subsidiary" shall mean with respect to a person, another  
person owned as to at least fifty percent (50%) of its equity or other ownership interests; and  
29 controlled, by the first person.

31 1.2 "Agent" shall mean any company or entity through which Licensee and/or any  
Affiliate and/or any Third Party Licensee produces and/or markets Licensed Products on behalf of  
33 such person.

35 1.3 "Chimera" shall mean any synthetic oligonucleotide of DNA and RNA and/or  
derivatives intended to create a specific alteration in a target sequence of the genome of a cell, the  
37 manufacture, use or sale of which falls within the disclosure and/or claims of U.S. patent no.  
5,565,350 or any application or patent claiming the same priority date as that patent.

39 1.4 "Commercial Sale" shall mean a sale of any Licensed Product by Licensee or any  
41 Affiliate, Agent or Third Party Licensee to an end user customer of that Licensed Product.

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1.5 "Confidential Information" shall mean all information, in whatever form, which is disclosed by either party to the other prior to or subsequent to the Effective Date of this Agreement.

1.6 "Enabling Technology" shall mean, Intellectual Property which covers the manufacture, design, delivery and/or use of Chimera, molecules, compounds, drugs, adjuvants, proteins or other material that are used in conjunction with Chimera to alter, modify or deliver DNA and/or RNA, in each case to the extent that they facilitate: (i) dissolution or and/or suspension of the Chimera (and associated agents); (ii) transit of Chimera across cell walls and membranes (including, without limitation, cellular, nuclear, mitochondrial and chloroplast membranes); (iii) protection of Chimera from degradation or inactivation; (iv) target localization to, and accurate pairing of, a target with Chimera; (v) enzyme localization to a Chimera target pairing site; (vi) using or working in conjunction with Chimera to make accurate base changes in genomic and/or target sequences as predicated in any given Chimera design; and any other Intellectual Property which covers aspects of the Chimera design, manufacture, storage, use, mixture, and/or manipulation and/or that improves and/or changes in any way characteristics of such Chimera to locate the desired target and attract or operate on or with appropriate cellular components and/or functions to perform efficient and specific base changes (including without limitation insertions or deletions); but shall not include any such Intellectual Property which is specific to the DNA and/or RNA sequence being altered, modified or delivered.

1.7 "Field" means Licensed Products that are Plants, but excludes Pharmaceutical Use.

1.8 "Full-Price Units" shall mean Units of Licensed Product that are invoiced to end user customers and are not Sample Units or Replant Units.

1.9 "Intellectual Property" shall mean without limitation, all patents and patent applications (including utility patents and plant patents and applications for utility patents and plant patents), patentable inventions, plant variety certificates and applications, know-how, trade secrets, techniques and ideas and all technical documentation and/or information which further includes DNA and/or RNA sequences and/or modifications thereof and genes, oligos and proteins, and associated methods and all applications therefor.

1.10 "Kimeragen Licensors" shall mean TJU and any other person that licenses any part of the Licensors Technology to Licensors, including licensors of Third Party Enabling Technology.

1.11 "License Revenues" shall mean all revenues received from Third Party Licensees including, but not limited to, license fees, royalties, and milestone payments.

1.12 "Licensed Genetic Material" shall mean genetic material that has been altered, identified, modified or created (in that generation or an earlier generation) by the use (in whole or

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1 in part) of the Licensor Technology which is the subject of any Patent Right or of any other Licensor  
Technology which Licensee would not have the right to use but for this License.

3 1.13 "Licensed Products" shall mean products containing Licensed Genetic Material.

5 1.14 "Licensee Enabling Technology" shall mean all Enabling Technology owned by or  
7 licensed to Licensee or any Affiliate.

9 1.15 "Licensee Product Rights" shall mean any patent or patent application (including any  
11 utility patent or plant patent and any application for a utility patent or plant patent), Plant Variety  
Certificate or plant variety application owned or licensed by Licensee or any Affiliate which covers  
any Licensed Product or the sale, use, development or production of any Licensed Products.

13 1.16 "Licensor Technology" shall mean all Enabling Technology owned by or licensed  
15 to Licensor, including Third Party Enabling Technology. Licensor Technology shall further include  
all Patent Rights that cover Licensor Technology.

17 1.17 "Patent Rights" shall mean patents and patent applications listed in Exhibit A and all  
19 foreign counterparts thereof in the Territory, including all divisionals and continuations,  
21 continuations in part, additions, confirmations, renewals, extensions, reexaminations and reissues  
of patents and patent applications and their equivalents covering the Licensor Technology. Patents  
23 and patent applications and all foreign counterparts thereof in the Territory covering Third Party  
Enabling Technology, including all divisionals and continuations, continuations in part, additions,  
confirmations, renewals, extensions, reexaminations and reissues of patents and patent applications  
25 and their equivalents shall be deemed to be included in Exhibit A and to be part of the Patent Rights  
upon (i) becoming Third Party Enabling Technology and (ii) being licensed to Licensee hereunder.

27 1.18 "Patent Term" shall mean the period from the Effective Date until the expiration of  
29 the last to expire of any Patent Right having a valid claim which, but for this Agreement, Licensee  
would infringe by any activity permitted under this Agreement.

31 1.19 "Plants" shall mean multicellular rooted organisms containing chlorophyll and  
33 cellulose cell walls.

35 1.20 "Pharmaceutical Use" shall mean (a) manufacture, synthesis and/or metabolism in  
Plants (naturally or through genetic engineering) of compounds, precursors or other products with  
37 pharmaceutical applications as active ingredients; (b) all harvesting, extraction, refinement,  
production and sale of such pharmaceutical compounds, precursors or other products; and (c) all uses  
39 related to such pharmaceutical compounds, precursors or other products that are or would be  
regulated by the FDA Centers of Biological Evaluation and Research (CBER), Drug Evaluation and

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1 Research (CDER), Devices and Radiologic Health (CDRH) and Veterinary Medicine (CVM) in any  
3 species, and all foreign agencies regulating similar subject matter.

5 1.21 "Replant Units" shall mean Units of Licensed Product that are invoiced to end user  
7 customers at a reduced value for use to replace defective seed and/or seed loss due to flooding or  
9 other damage or quality problems.

11 1.22 "Sample Units" shall mean Units of Licensed Product that are invoiced at no value  
13 to end user customers for promotional purposes.

15 1.23 "Territory" shall mean the world.

17 1.24 "Third Party Enabling Technology" shall mean Enabling Technology owned or  
19 controlled by other licensees of the Licensor Technology that has been licensed to Licensor.

21 1.25 "Third Party Licensee" shall mean any company or entity, other than an Agent, that  
23 is licensed by Licensee and/or any Affiliate to Licensed Products or Licensed Genetic Material.

25 1.26 "Unit" shall have the meaning specified on Exhibit B.

27 1.27 "University" shall mean a university or other institution of higher education that is  
29 exempt from taxation under clause 501(a) of the Code (26 U.S.C. § 501(a)).

## 31 ARTICLE 2--LICENSE

33 2.1 Grant of License. Licensor hereby grants Licensee, upon the terms and conditions  
35 set forth in this Agreement, a royalty-bearing non-exclusive license under the Licensor Technology  
37 in the Territory to use Licensor Technology to conduct product development in the Field and to  
39 make, use, sell, import and export Licensed Products in the Field.

2.1.1 Licensee shall have the right to subcontract to Licensee's Subsidiaries and to  
Universities activities relating to the development, and to Licensee's Affiliates and Agents  
activities relating to the growing and marketing of Licensed Products, for the purposes of  
conducting Licensee's ongoing business. Such subcontracting shall not be considered a  
sublicense, and Licensee shall be responsible for such activities as if they were the activities  
of Licensee. Without limiting the foregoing, Licensee shall not subcontract any development  
work to any Subsidiary or University without requiring that the results of such development,  
if Enabling Technology, be subject to the license provided in Section 2.3.1.

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1           2.1.2 Licensee shall have the right to sublicense its rights hereunder for activities  
3 within the Field to Third Party Licensees but only to the following extent:

5                   (a) Licensee shall have the right to sublicense Licensed Genetic Material  
7 alone;

9                   (b) Licensee shall have the right to sublicense Licensed Genetic Material  
11 incorporated into other germ plasm; and

13                   (c) Licensee shall have the right to sublicense the growing and marketing  
15 of Licensed Products.

17           2.1.3 Licensee shall have the right to assign in part the license granted in Section  
19 2.1 to Pioneer Overseas Corporation ("POC") within the Field; provided that POC shall not  
21 further assign any rights under this Agreement other than together with, and not separate  
23 from, any other permitted assignment by Licensee in accordance with Section 8.4; and  
25 provided further that Licensee gives Licensor prompt written notice of the exercise of that  
right and the scope of the grant. Notwithstanding any exercise by Licensee of rights under  
this Section 2.1.3, Licensee shall be responsible for activities of POC as if they were  
activities by Licensee.

27           2.1.4 No license is granted hereunder for sale of Licensed Products as commodity  
29 grain, except to the extent that seed grain is discarded as a result of obsolescence or quality  
defects.

31           2.2 Improvements. During the term of this Agreement, the parties shall promptly disclose  
33 to each other in reasonable detail, including available written protocols of the methodology and of  
35 the materials used, and any available replicated data obtained, any newly developed or controlled  
37 Licensee Enabling Technology or Licensor Technology.

39           2.3 Further Licenses.

41           2.3.1 Licensee hereby grants to Licensor a worldwide license (substantially on the  
terms contained in this present license but: (i) excluding payment obligations other than  
Section 3.8, and recognizing the respective roles of the parties to the license back, and  
(ii) limiting use solely to applications in conjunction with Chimera) to Licensee Enabling  
Technology on a non-exclusive basis for all applications with the right to grant sublicenses  
(which may further include the right to grant sublicenses). Licensor shall sublicense  
Licensee Enabling Technology of a specific type to each particular licensee only to the extent  
(i) such licensee has the similar obligation to grant Licensor a license (including the right to  
grant sublicenses) under its rights in that particular type of Enabling Technology; (ii)

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1 Licensee is granted rights to such Enabling Technology as part of the Licensor Technology;  
2 and (iii) such licensee has covenanted substantially as provided in Section 2.3.2. Without  
3 limiting Section 2.3.2, such license shall not grant any rights to Intellectual Property owned  
4 or licensed by Licensee or any Affiliate prior to the Effective Date.

5  
6 2.3.2 Licensee covenants for the benefit of Licensor and Licensor's other licensees  
7 of Enabling Technology in the Field (who may enforce this provision directly) not to assert  
8 any patent owned or controlled by Licensee to prevent use of any Enabling Technology in  
9 conjunction with Chimera pursuant to a license from Licensor in the Field.

11 2.3.3 In negotiating with a third party any potential license for Enabling  
12 Technology from that third party that is exclusive or would otherwise prevent that third party  
13 or Licensee from granting rights to Licensor or other licensees of Enabling Technology,  
14 Licensee shall either (i) obtain the right to sublicense such Enabling Technology to Licensor  
15 and its other licensees of Enabling Technology, or (ii) include Licensor in such negotiations  
16 so that Licensor may seek to obtain rights for use with Chimera.

17  
18 2.4 Protection of Technology. Licensee shall not use any Licensor Technology, nor shall  
19 Licensor use any Licensee Enabling Technology, for any purpose other than as provided in this  
20 Agreement, unless such technology shall come into the public domain.

21  
22 2.5 Acknowledgment of Rights. Licensee acknowledges that Licensee's right to use the  
23 Licensor Technology arises only out of the licenses granted under this Agreement. All Licensed  
24 Products shall bear a patent notice on the label to the extent, if any, as may be required under the  
25 laws of the country in Territory in which the Licensed Products are sold.

## 27 28 ARTICLE 3--PAYMENT

29  
30 3.1 Payment. In consideration of the rights granted herein, Licensee shall pay to  
31 Licensor:

32  
33 3.1.1 A one-time non-refundable payment of one hundred thousand dollars  
34 (US\$100,000) upon the date of execution of this Agreement. This payment shall be  
35 attributable in part to issued patents as set forth in Exhibit A (ninety thousand dollars  
36 (US\$90,000)) and in part to know-how, and rights to continuations of such patents; and  
37 rights in future patents and other technology as set forth in Exhibit A and elsewhere in this  
38 Agreement (ten thousand dollars (US\$10,000));  
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1           3.1.2 A one-time non-refundable payment of nine hundred thousand (US\$900,000)  
3       for the conduct of research on behalf of Licensee in pursuit of Milestone IA (as provided in  
      Section 3.1.3 below);

5           3.1.3 Further one-time non-refundable payments of: (i) five hundred thousand  
7       dollars (US\$500,000) for the conduct of research on behalf of Licensee in pursuit of  
9       Milestone II upon the achievement of Milestone IA (as provided below); (ii) five hundred  
11       thousand dollars (US\$500,000) for the conduct of research on behalf of Licensee in pursuit  
13       of Milestone II upon the achievement of Milestone IB (as provided below); and (iii) one  
15       million dollars (US\$1,000,000) as a bonus for the successful completion of Milestone II. All  
      payments will be payable within thirty (30) days of the earlier of (a) achievement by  
      Licensee of each Milestone; or (b) written notification to Licensee by Licensor of the  
      achievement of each milestone, together with written protocols of the methodology and  
      materials used, and data which has been replicated at least once demonstrating that such  
      Milestone was reached;

17               (a) Milestone IA: proof of principle of Licensor Technology in a dicot  
19       plant species as demonstrated by a genomic sequence altered as predicated by a  
      specific Chimera design;

21               (b) Milestone IB: proof of principle of Licensor Technology in a  
23       monocot plant species as demonstrated by a genomic sequence altered as predicated  
      by a specific Chimera design; and

25               (c) Milestone II: creation of any Plant using or incorporating (in any  
27       generation) Licensor Technology and/or Licensee Enabling Technology as  
      demonstrated by the trait of the target altered as predicated by a specific Chimera  
      design and such trait is transmitted to at least its next generation progeny;

29       provided that if any such milestone is reached prior to Licensee executing this Agreement,  
31       the payment due for such milestone under this Section 3.1.3 shall be added to the up-front  
33       fee payable under Sections 3.1.1 and 3.1.2;

35           3.1.4 A further non-refundable payment of one million dollars (US\$1,000,000)  
37       upon the earlier of (i) filing of the first patent application (including any application for a  
39       utility patent or plant patent) or plant variety protection application in the Territory with  
      respect to any Licensed Products by Licensee or any Affiliate, or designating Licensee or any  
      Affiliate as assignee; or (ii) the first Commercial Sale;

41           3.1.5 A royalty per Unit of Licensed Products as specified in Exhibit B for use of  
      Licensor Technology in developing Licensed Products, which the parties agree for

1 convenience shall be structured as set out in Section 3.2. Notwithstanding anything to the  
3 contrary herein, in a series of transactions, only the Commercial Sale shall be treated as  
royalty bearing for the purpose of this Agreement; and

5 3.1.6 Royalties as provided in Section 3.4.

7 3.2 Royalty Conditions. Royalties shall:

9 3.2.1 be payable for the duration of the Patent Term:

11 (a) on Commercial Sales by Licensee and/or Affiliates and/or Agents of  
13 Licensed Products that are Full-Price Units or Sample Units, and

15 (b) uses of Licensed Products by Licensee and/or any Affiliate and/or any  
Agent unless the result of that use is a subsequent royalty-bearing sale under Section  
17 3.2.1(a), with royalty to be payable on the Licensed Products used in the same  
amount as if such Licensed Products had been the subject of a Commercial Sale of  
19 Full-Price Units

in both cases where such Licensed Products are either :

21 (i) at the time and place of development, covered by a pending or issued  
23 claim in the Patent Rights; or

25 (ii) at the time and place of either use, production or sale, covered by a  
27 pending or issued claim in the Patent Rights;

29 3.2.2 be payable for a period of five (5) years after the Patent Term (not as post-  
expiration royalties but for use of Licensor Technology during the Patent Term to develop  
31 Licensed Products, the payment for which has been agreed for convenience shall be  
measured by sales of Licensed Products), on Commercial Sales by Licensee and/or Affiliates  
33 and/or Agents of those Licensed Products described in Section 3.2.1(i) which are Full-Price  
Units or Sample Units and uses under Section 3.2.1(b) of those Licensed Products described  
35 in Section 3.2.1(i); provided that, at the time of production, sale or use, the Licensed Product  
or its production, sale or use, is covered by a Licensee Product Right;

37 3.2.3 accrue upon the sale, as determined by U.S. GAAP, of Licensed Products by  
Licensee and/or Affiliates and/or Agents or upon use pursuant to Section 3.2.1(b);

39 3.2.4 be due and payable within sixty (60) days of the end of each fiscal year of the  
41 Licensee in U.S. dollars. Subject to the next sentence, any and all withholding taxes levied

1 on account of royalties or milestones accruing under this Section 3 shall be paid by Licensee,  
2 on behalf of Licensor. If laws or regulations require withholding of said taxes on royalties,  
3 such taxes may be deducted from such remittable royalty only if (i) such taxes will be paid  
4 by Licensee, in the name of Licensor to the proper taxing authority, and (ii) proof of payment  
5 and any other documentation required by Licensor to obtain credit for any such payment  
6 from the U.S. tax authorities shall be sent to Licensor no later than forty-five (45) days  
7 following December 31st of each reporting year. Taxes may not be withheld from the  
8 amount of milestone payments made to Licensor.

9  
10 3.3 At any time after the first anniversary of the Effective Date, but not more frequently  
11 than once in any twenty-four (24) month period, Licensee may give written notice to Licensor that  
12 it desires to negotiate a lump sum payment in lieu of royalties payable under Sections 3.1.5 and 3.4  
13 for any one or more Licensed Products. Within thirty (30) days of receiving such notice, Licensor  
14 shall commence negotiations with Licensee to determine an appropriate amount for such lump sum  
15 payment for each Licensed Product nominated by Licensee. If the parties are unable to agree on an  
16 amount for each Licensed Product nominated by Licensee within sixty (60) days of the  
17 commencement of such negotiations, Licensor may notify Licensee that it will not accept a lump  
18 sum payment in lieu of royalties at that time, at all, or for those Licensed Products with respect to  
19 which no agreement was reached.

#### 20 3.4 License Revenues.

21  
22 3.4.1 Licensor shall be entitled to share in all License Revenues derived by  
23 Licensee and/or any Affiliate. Where License Revenues are payable to Licensee in cash or  
24 equivalent legal tender pursuant to Section 2.1.2(a), Licensee shall pay Licensor fifty per  
25 cent (50%) of such License Revenues within thirty (30) days after the calendar quarter in  
26 which such License Revenues are received.

27  
28 3.4.2 In the event that Licensee proposes to:

29  
30 (a) barter or trade a sublicense under Section 2.1.2 (including any license  
31 to an Agent for purposes other than producing and/or marketing any Licensed  
32 Product for or on behalf of Licensee and/or any Affiliate) in return for other  
33 technology, or Licensee shall otherwise receive non-cash consideration for such  
34 sublicense (in whole or in part); and/or

35  
36 (b) sublicense pursuant to Sections 2.1.2(b) or (c),

37  
38 then Licensee shall not proceed with such sublicense without Licensor's consent, which shall  
39 be predicated upon agreement between the parties on an appropriate fee payable by Licensee  
40 to Licensor with respect to such license. Such fee shall be equal to the proportional value  
41

1 of the Licensed Genetic Material as compared to any other germ plasm licensed, expressed  
2 as a percentage and divided by two (2), provided that in no event shall the amount of royalty  
3 payable to Licensor be less than that amount which would be payable pursuant to Section  
4 3.1.5 on the sale or use of the Licensed Products sold or used by the Third Party Licensee.  
5 An example of such a calculation is provided on Exhibit C. Such percentage shall be applied  
6 to the value of any such barter or trade plus any cash or cash equivalent forming part of the  
7 consideration, payable within sixty (60) days of the earlier of entry into such license or  
8 conclusion of the procedure of Section 8.13.2, or with respect to ongoing payments, within  
9 sixty (60) days of receipt of payment by Licensee. In the event that the parties are unable to  
10 agree on an appropriate fee within sixty (60) days of Licensee notifying Licensor of the  
11 proposed license, Licensee may enter into the, license subject to the procedure of  
12 Section 8.13.2 to determine the fee payable to Licensor.

13 3.4.3 Licensee shall be responsible for monitoring and reporting on the sales of  
14 Affiliates, Third Party Licensees and Agents to the extent information is necessary for the  
15 calculation of royalties, and shall be responsible for all royalties due to Licensor.

16 3.5 Reports. Licensee shall provide royalty reports to Licensor together with each  
17 payment made under Sections 3.1.5 and 3.4. Such reports shall contain all information relating to  
18 Licensee's sales as well as sales by Affiliates, Third Party Licensees and Agents and License  
19 Revenues received, as reasonably necessary to verify amounts due under this Agreement, including:  
20

21 (a) a listing of all Licensed Products qualifying for royalties sold or used by  
22 Licensee, Affiliates, Third Party Licensees and Agents, sorted by individual product, crops,  
23 species, country and number of Units;

24 (b) all License Revenues received, sorted by Third Party Licensee, license fees,  
25 royalties and milestone and other payments;

26 (c) royalty calculation; and

27 (d) royalties due and payable.

28 3.6 Records. Licensee shall also keep full and accurate records at its principal place of  
29 business in the United States or, if Licensee has no principal place of business in the United States,  
30 at a location agreed upon in writing by the parties, of all Licensed Products developed, used, grown,  
31 distributed, or sold by Licensee, Affiliates, Third Party Licensees and Agents and any other records  
32 reasonably necessary to enable verification of reports provided under Section 3.5 .  
33

34 3.7 Audit. Records kept by Licensee pursuant to Section 3.6 shall be open, at reasonable  
35 times during business hours, to an inspection by a mutually agreed upon Certified Public  
36 Accountant.



1 Accountant, at Licensor's expense, for the purposes of verifying Licensee's royalty payments under  
this Agreement. If a shortage of greater than five percent (5%) is established in any payment due  
3 hereunder, Licensee shall reimburse Licensor for the cost of such inspection and promptly pay such  
overdue amount together with interest at the rate specified in Section 3.9. In addition, in the event  
5 that Licensee or a Subsidiary has commercialized a Licensed Product but Licensee has failed to  
make any royalty payments hereunder with respect to such Licensed Product, then Licensee shall  
7 promptly pay all such past due royalties plus interest of twenty-five percent (25%) per annum,  
calculated from the date such royalties became due until the date such royalties are paid.

9  
3.8 Third Party Royalties. In any case where use of the Licensor Technology is in the  
11 future subject to a royalty (whether lump-sum or payable by reference to sales) to a third party (other  
than a royalty to TJU with respect to Licensor Technology licensed by Licensor as at the date  
13 hereof), then Licensee shall, in addition to the royalty specified in Section 3.1, be responsible for  
payment to Licensor of a further amount equal to the royalty payable to the third party with respect  
15 to Licensee's use under this Agreement, except to the extent Licensee shall be separately licensed  
by that third party with respect to such use. In any case where use of the Licensee Enabling  
17 Technology is in the future subject to a royalty (whether lump-sum or payable by reference to sales)  
to a third party, Licensor shall be responsible for payment to Licensee of an amount equal to the  
19 royalty payable to the third party with respect to Licensor's or its licensees use of such Enabling  
Technology under this Agreement, except to the extent Licensor or any of its licensees shall be  
21 separately licensed by that third party with respect to such use.

23 3.9 Late Payments. All payments to be made by the Licensee to the Licensor hereunder  
shall bear interest at the prime or equivalent rate as quoted by Citibank N.A., New York, New York,  
25 on the day the payment is overdue plus two percent (2%) per annum from the date payment becomes  
overdue, until paid.

27  
3.10 Royalty Structure. Licensee represents and warrants that: (i) it currently markets its  
29 seed products through a system pursuant to which all seed produced by Licensee, Affiliates and  
Agents is invoiced by Licensee and sold to the end user customer (*i.e.*, the grower of commodity  
31 grain); (ii) seeds are either sold as Full-Price Units, Sample Units or Replant Units and not in any  
other manner; (iii) seeds are not sold as foundation stock other than to Affiliates or Agents where  
33 seed produced is either a royalty-bearing use or Commercial Sale under this Agreement; and (iv)  
seeds are not sold for purposes other than growing commodity grain except as provided in Section  
35 2.1.4. The parties acknowledge that it is the intention of this Agreement that Licensor receive  
royalties and payments based on Licensee's business as presently structured of the amounts set forth  
37 herein. In the event the structure of Licensee's business changes such that Licensee receives  
revenues in a different manner, the parties shall restructure the royalty and payment mechanisms set  
39 forth herein to achieve equivalent royalty and payment amounts using, if applicable, a royalty  
structure offered to Licensee pursuant to Section 7.6. In the absence of agreement within sixty (60)

1 days of commencing negotiations hereunder, any such dispute shall be resolved pursuant to Section  
2 8.13.2.

3  
4 3.11 Effect on Agreement. This Agreement shall not become effective until payments as  
5 described in Sections 3.1.1 and 3.1.2 have been made.

#### 7 ARTICLE 4--PATENT INFRINGEMENT

9 Licensee shall notify Licensor promptly of any action, claim or threat of patent infringement suit,  
10 either oral or written, or the commencement of any such patent infringement suit against Licensee  
11 relating to the Licensor Technology. The parties shall cooperate in the development and execution  
12 of a strategy to defend against any such action against Licensee, Licensor or other licensee of  
13 Licensed Technology in the Field by a third party. Each party shall also notify the other promptly  
14 of any infringement, in the Field, of the Patent Rights by a third party of which that party becomes  
15 aware. In the event of a suspected infringement of the Patent Rights by a third party with respect to  
16 Licensed Products being developed or commercialized by Licensee, both parties will consult with  
17 each other and any other affected licensees of Licensor to determine the appropriate strategy to  
18 attempt to prevent such infringement.

#### 20 ARTICLE 5--WARRANTIES & INDEMNITIES

##### 22 5.1 Warranties.

23  
24 5.1.1 Licensor represents and warrants that it has full right, power and authority to  
25 enter into this Agreement and that the terms of this Agreement do not conflict with any other  
26 contractual obligations it has.

27  
28 5.1.2 Licensor represents and warrants that, as of the execution date, it knows of  
29 no Intellectual Property that would prevent Licensee from practicing the whole of the  
30 Licensor Technology as described in U.S. Patent No. 5,565,350. Licensor is aware of  
31 numerous patents covering particular nucleic acids, nucleotides, intermediates, gene  
32 fragments and other aspects of genetic technology.

33  
34 5.1.3 Licensor represents and warrants that it has the freedom to enter into this  
35 Agreement and the right to provide the rights and license contemplated in Section 2.1.

36  
37 5.1.4 Licensor represents and warrants that, as of the Effective Date, Licensor has  
38 disclosed to Licensee all known, and knows of no other, royalty obligations pursuant to  
39 Section 3.8.

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5.2 No Other Warranties. Neither party makes any warranty or representation with respect to the Enabling Technology, nor is either party in any way responsible for the utility of any Enabling Technology. BOTH PARTIES HEREBY EXPRESSLY DISCLAIM ANY AND ALL WARRANTIES AND REPRESENTATIONS, EXPRESS OR IMPLIED, ARISING BY LAW OR CUSTOM, WITH RESPECT TO THE ENABLING TECHNOLOGY, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, PATENTABILITY OR NON-INFRINGEMENT. NEITHER PARTY IN ANY WAY PROMISES THAT THE LICENSOR TECHNOLOGY SHALL PRODUCE ANY PARTICULAR RESULTS, PRODUCTS OR PROFITABILITY.

5.3 Indemnities. Licensee hereby waives any claim against Licensor and the Kimeragen Licensors and agrees to indemnify, defend, and hold harmless Licensor and the Kimeragen Licensors and their respective directors, officers, employees and agents, and in the case of the Kimeragen Licensors, their directors, trustees, officers, agents and employees and those of any associated University, from all liabilities, demands, damages, expenses and losses (including without limitation for death, personal injury, illness or property damage, and including reasonable attorneys' fees) arising out of or in connection with Licensee's or its transferee's actions under this Agreement (collectively, the "Indemnified Losses"), including without limitation Indemnified Losses resulting from any exercise or use by Licensee or its transferees of Patent Rights, and any use, sale, or other disposition of Licensed Products by Licensee or its transferees and any claim that Licensee's use, sale, or other disposition of Licensed Products infringes or violates any patent or other Intellectual Property rights. The indemnification rights contained herein are in addition to all rights which Licensor and/or the Kimeragen Licensors may have at law or in equity. Licensee hereby agrees that the Kimeragen Licensors are entitled to enforce this Section 5.3 directly against Licensee. As used in this Section 5.3, Licensee includes its Affiliates, Subsidiaries, contractors and sub-contractors. Notwithstanding the foregoing, the indemnities provided by Licensee herein shall not apply with respect to Licensor to the extent the indemnified losses would be or are covered by the foregoing Licensor's warranties.

**ARTICLE 6--TERM**

6.1 Term Of Agreement. This Agreement shall become effective upon signature by both parties and payment by Licensee of the sums specified in Sections 3.1.1 and 3.1.2. Subject to Section 6.4 and Article 3, and to earlier termination as provided herein, all rights and obligations hereunder shall expire upon the conclusion of the Patent Term. Subject to Section 6.3, upon expiration of the royalty obligation pursuant to Article 3, Licensee shall have a fully paid up license to all Licensor Technology licensed during the term of this Agreement without further obligation to Licensor.

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1           6.2   Early Termination.

3           6.2.1   Subject to Section 6.3, Licensee shall have the right to terminate this  
5   Agreement by at least sixty (60) days written notice to Licensor.

7           6.2.2   Subject to Section 6.3, either party may terminate this Agreement with  
9   immediate effect by written notice to the other party, if the other party commits a breach of  
any material obligation under this Agreement and fails to remedy such breach within sixty  
(60) days after notice of such breach.

11          6.3   Effects of Termination. Termination or expiration of this Agreement shall not affect  
13   the continued enforceability of Sections 2.3, 5.3, 7.2, 7.4, 7.5 and 8.13, and Article 3 (with respect  
to any sale or use of Licensed Products, until the date on which the last of any royalty payments  
payable under Article 3 have been paid).

15  
17                           **ARTICLE 7--OTHER AGREEMENTS**

19          7.1   Supply of Chimera. Licensee may obtain its supply of Chimera solely from:  
21   (i) Licensor under terms to be negotiated between the parties; (ii) vendors that are licensed by  
Licensor to produce Chimera or (iii) from its own production.

23          7.2   Confidentiality. Each party shall:

25               (a)   maintain the Confidential Information of the other party in confidence and  
27   refrain from disclosing any part of such Confidential Information to any person or entity  
other than to its employees, consultants, subcontractors or sublicensees whose duties or  
rights justify the need to know such Confidential Information;

29               (b)   not to make any use of the Confidential Information other than for the purpose  
31   of carrying out duties and obligations and exercising rights under this Agreement;

33               (c)   to take all reasonable steps to protect the Confidential Information against  
35   disclosure, misuse, loss and theft, which steps include the execution by all such persons of  
written agreements containing obligations of confidentiality, restricted disclosure and limited  
use relative thereto consistent with this Section 7.2 prior to disclosure of Confidential  
37   Information to them; and

39               (d)   in the event that a third party wishes to evaluate Confidential Information in  
41   connection with a proposed business transaction with a party, disclose only Enabling  
Technology developed by Licensee hereunder to that third party as is necessary to conduct

1 such evaluation, provided that prior to disclosure such third party executes a written  
3 agreement prohibiting use of the Confidential Information for any reason other than  
5 evaluation of such transactions and containing obligations of confidentiality consistent with  
this Section 7.2.

7 The provisions of this Section 7.2 shall not apply to any part of the Confidential Information  
disclosed by one party to the other (a) which is agreed in writing by the disclosing party to be  
9 excluded; or (b) which the receiving party can show was known to or developed by it prior to  
Confidential Information first being received by it from, or disclosed to it by, the disclosing party;  
11 or (c) which is public knowledge, or becomes public knowledge in the future, other than through acts  
or omissions of the receiving party in breach of this Agreement; or (d) which is lawfully obtained  
13 by receiving party from sources independent of the disclosing party who have a lawful right to  
possess and disclose such Information; (e) which it is necessary for the receiving party to disclose  
15 in order to comply with any applicable law or if required to do so by order of any court or any other  
judicial or administrative body, provided that prior to making such disclosure the receiving party  
17 gives the disclosing party notice of the requirement of disclosure and the information to be disclosed;  
or (f) which is to be included in any patent application, provided that prior to filing any such patent  
19 application, the party proposing to file such application shall provide a copy to the other party and  
not file such application until the other party shall have consented, such consent not to be  
unreasonably withheld or delayed.

21 7.3 Press Releases. Prior to any press release concerning the execution of this  
23 Agreement, its terms and conditions, or any subsequent event under this Agreement which either  
party considers newsworthy, both parties shall agree on the content and timing, such consent not to  
25 be unreasonably withheld.

27 7.4 Regulatory Approvals. The parties shall provide to one another (at no cost) all  
materials, data and information in their possession needed to seek and obtain regulatory approvals  
29 necessary for the use and sale of Licensed Products. Neither party shall use any regulatory  
information and/or packages developed by the other for the benefit of a third party, except that  
31 Licensor shall be permitted to use such information to establish master files for filing with regulatory  
agencies. Licensee shall comply with all regulatory requirements relating to the Licensed Products  
33 and shall take all reasonable or required steps to ensure that the Licensed Products are safe and  
lawful.

35 7.5 Product Liability Insurance. Licensee shall obtain and maintain commercial general  
37 liability insurance, including commercial liability, product liability and completed operations  
insurance coverage in a minimum amount of five million dollars (\$5,000,000) per loss including  
39 coverage for contractual liability. Licensor and the Kimeragen Licensors that are Universities and  
their respective officers, directors, trustees, members of governing boards and employees will be  
41 named insureds under all such insurance. Such insurance shall also provide that Licensor and the

1 Kimeragen Licensors that are Universities be given notice of any modification thereof and at least  
2 ten (10) days prior written notice of cancellation or termination and the reason therefor. A certificate  
3 of insurance evidencing such coverage will be provided to Licensors and, upon each annual  
4 anniversary of this Agreement, Licensee shall provide written confirmation issued by the insurer or  
5 an independent insurance agent confirming that insurance is maintained in accordance with the  
6 above requirements. Subject to Licensee obtaining consent from the Kimeragen Licensors that are  
7 Universities, Licensee may elect to be self-insured in accordance with reasonable business practices,  
8 provided that the above requirements are met.

9  
10 7.6 Equivalent Royalty Structure. Licensors undertake to Licensee that it will not offer  
11 any third party licensee of Licensors Technology in the Field any up-front payment or milestone  
12 payment lower than those set out in Sections 3.1.1, 3.1.2, 3.1.3 and 3.1.4; or any royalty rate lower  
13 than that set out in Section 3.1.5 and Exhibit B without offering such terms to Licensee to the extent  
14 applicable to Licensee's business structure.

15  
16 7.7 Committee. A committee of not more than six (6) persons comprising an equal  
17 number of representatives of each party shall be formed (the "Committee") and shall meet not less  
18 than once each calendar year to discuss research, progress toward achievement of the milestones set  
19 out in Sections 3.1.3 and 3.1.4 and commercialization of Licensed Products. At such meeting,  
20 Licensee shall provide to Licensors, in a document marked "confidential," a description of the  
21 projects to which Licensee or its Subsidiaries or Universities subcontracted by Licensee have applied  
22 Chimera, a description of Licensed Products developed by Licensee or its Subsidiaries, and a  
23 notification of any such Licensed Products that have been commercialized.

24  
25 7.8 Trademarks and Use of Names. Neither party shall have any right to use any  
26 trademark or trade name of the other without the other party's prior written consent, and then subject  
27 to such terms and conditions as may be agreed to in writing by the parties. If Licensee develops a  
28 Licensed Product for commercialization, the Committee shall consider in good faith whether use of  
29 Licensors' trademarks or a description of Licensors Technology and/or a particular aspect thereof in  
30 marketing brochures, literature and labeling for any Licensed Product is appropriate and desirable,  
31 and the appropriate payment and other terms for such trademark use. Neither party shall use the  
32 name of the other, nor shall Licensee use the name of any Kimeragen Licensors, or the names of any  
33 of their respective staff members, employees or students or any adaptation thereof in any advertising,  
34 promotional or sales literature to the extent such use might imply a relationship between the parties,  
35 or endorsement by either party or any Kimeragen Licensors of any act or thing or any product or  
36 method described in such material, without the prior written consent of the other party and the  
37 Kimeragen Licensors where applicable, which consent shall not be unreasonably withheld.

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1           8.9    Interpretation. The headings contained in this Agreement are for convenience only  
and shall not affect the interpretation of this Agreement. In this Agreement, the word "including"  
3 shall be deemed to be followed by "without limitation", the words "hereof" and "herein" and  
"hereunder" refer to this Agreement as a whole, and the singular includes the plural and vice versa.

5           8.10   Notices. Notices shall be given by first class mail, by Federal Express or other  
7 recognized courier requiring signature on receipt, and shall be addressed to the other party at the  
address set forth below (or at such address as a party may specify by notice to the other):

9                       If to Licensee: Pioneer Hi-Bred International Inc.

11                       7300 NW 62nd  
P.O. Box 1004  
13                       Johnson, IA 50131-1004

15                       Attention: Director, Research Technology Services

17                       Telephone: (515) 270-3600

Telecopy: (515) 253-2478

19                       If to Licensor: Kimeragen, Inc.

21                       300 Pheasant Run  
Newtown, PA 18940

23                       Attention: President

25                       Telephone: (215) 504-4444

Telecopy: (215) 504-4545

27           8.11   Governing Law and Jurisdiction. Pursuant to Section 5-1401 of the New York  
General Obligations Law, this Agreement shall be governed by and construed in accordance with  
29 the laws of the State of New York without giving effect to any choice of law or conflict of law  
provision or rule that would cause the application of the laws of any jurisdiction other than the State  
31 of New York.

33           8.12   Counterparts. This Agreement may be executed in one or more counterparts, each  
of which shall be deemed an original, but all of which together shall constitute one and the same  
35 instrument.

37           8.13   Dispute Resolution.

39                       8.13.1 The parties shall work together to remedy any difficulties which may arise in  
connection with this Agreement. All disputes arising out of this Agreement (other than  
41 disputes arising under Section 3.4 or 3.10, which shall be resolved in the manner described



1 below in Section 8.13.2) shall be referred to decision forthwith to a senior executive of each  
2 party who is, if possible, not involved in the dispute. If no agreement can be reached through  
3 this process within thirty (30) days of request by one party to the other to nominate a senior  
4 executive for dispute resolution, then either party hereto shall be entitled to bring  
5 proceedings relating to such dispute. Any and all such proceedings shall be brought in a  
6 court having jurisdiction over the parties in the County of Manhattan, New York.

7  
8 8.13.2 Any dispute arising under Section 3.4 of this Agreement which is not resolved  
9 between the parties within sixty (60) days of Licensee notifying Licensor of a proposed  
10 sublicense, or any dispute under Section 3.10 or Exhibit B, shall be referred to a partner of  
11 Arthur Andersen & Company in Philadelphia nominated by that firm (the "Arbitrator") for  
12 decision on (i) in the case of Section 3.4, which proposal for the fees payable to Licensor for  
13 such sublicense, (ii) in the case of Section 3.10, which revised payment structure, last put  
14 forward by the respective parties prior to the referral to arbitration is the most reasonable in  
15 the circumstances; or (iii) in the case of Exhibit B, which proposal last put forward by the  
16 respective parties prior to the referral to arbitration is the most reasonable in the  
17 circumstances. The Arbitrator shall have no discretion to make any determination other than  
18 a choice between the last proposal put forward by each of the parties. The parties may each  
19 present written material to the Arbitrator to support their last offer, provided such material  
20 is submitted to the Arbitrator within thirty (30) days of the matter being referred to the  
21 Arbitrator, and the parties will use their best efforts to ensure that the Arbitrator shall make  
22 a decision in the manner prescribed in this Section and notify the parties of such decision  
23 within thirty (30) days of the expiration of the time for the parties to submit written material,  
24 or earlier waiver by the parties of that right. The Arbitrator's decision shall be irrevocable  
25 and fully accepted by the parties, and Licensee shall pay the fee stipulated by the Arbitrator  
26 to Licensor with its next royalty payment after such decision. The party whose proposal was  
27 not chosen by the Arbitrator shall bear all reasonable costs incurred by both parties incurred  
28 in connection with such arbitration.  
29

\* \* \* \* \*

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1 IN WITNESS WHEREOF, the parties have caused their duly authorized representative to  
execute this Agreement as of the Effective Date.

3  
5 PIONEER HI-BRED INTERNATIONAL, INC.

7  
9 By: 

11 Name: Anthony J. Cavalieri

13 Title: Vice President

15 KIMERAGEN, INC.

17  
19 By: 

21 Name: Gerald L. Messerschmidt, M.D.

23 Title: President

1 **EXHIBIT A**

3 **Patent Rights<sup>1</sup>**

- 5
- 7 • United States Patent Number 5,565,350

- 9
- 11 • Foreign counterparts to '350 patent:

13

<u>Country</u>	<u>Application Serial No.</u>
Australia	13995/95
Canada	2,178,729
China, People's Republic	94194935.4
European Patent Convention	95905337.2
Korea (South)	703040/96
Japan	7-516367
New Zealand	278490

15

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19

21

- 23 • Saliwanchik, Lloyd & Saliwanchik docket no. KIM 100-P  
"Alteration of Plant and Yeast Genes"
- 25
- 27

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<sup>1</sup> Rights of Licensee in above listed patents and applications are limited to Enabling Technology in the Field and specifically exclude any right to practice any gene or sequence specific subject matter of any such patents or applications.

1 **EXHIBIT B**

3 **Royalty Calculations**

5 It is the intent of the Parties to have an equitable and auditable protocol for determining royalties.  
7 The following definitions shall be used:

9 "Adjusted Net Royalty Base" shall mean the Royalty Base, as annually adjusted by Royalty Base  
11 Adjustment Factor beginning in 1998 and compounded annually, less the Gross-to Net Factor. An  
13 example of the calculation of the Adjusted Net Royalty Base is set forth in Attachment B.2.

15 "Average Net Published Price" shall mean the average price in the U.S.A. for all non-transgenic  
17 "Performance Elite" products as published in the "Master Card" or equivalent.

19 "Base Year" shall mean the first year a Licensed Product that is a Qualifying Unit is sold in the  
21 U.S.A.

23 "Gross-to-Net Factor" shall mean the multiplier that is a proxy for the typical and traditional  
25 conversion of list price to invoice price for Full-Price Units (regardless of crop species), which  
27 equals 20%.

29 "Licensee Marketshare" shall mean Licensee Unit Sales divided by Total Market Size, expressed as  
31 a percentage.

33 "Licensee Unit Sales" shall mean Licensee's Unit sales of a particular crop species in the Qualifying  
35 Region as reported in commercially prepared reports, such as Merrits and Doans or any other  
37 publication agreed upon by the parties and listed on Attachment B.3, expressed in Units. In the  
39 absence of such reports, Licensee shall prepare a report documenting Licensee Unit Sales for  
41 agreement by the parties.

"Multiple-Use Royalty Rate" shall mean 0.70% or 1.0% for two (2) or more uses of Licensor  
Technology in one version of Licensed Product where Licensor's Marketshare is greater than 33.3%  
or less than 33.3%, respectively.

"Net Sales" shall mean the Adjusted Net Royalty Base times the number of Qualifying Units.

"Qualifying Region" shall mean the region composed of North America, South America and the  
European Union.

"Qualifying Units" shall mean Sample Units and Full-Price Units sold or used which create a royalty  
obligation pursuant to Section 3.2.

1 "Royalty Base" shall mean the royalty base and shall be one hundred and five dollars and ninety  
cents (US\$105.90) per Unit of Corn for 1997. For other crop species, the Royalty Base shall be  
3 calculated using the same protocol and formula as was used for Corn.

5 "Royalty Base Adjustment Factor" shall mean the average increase in the Average Net Published  
Price over a period starting in 1997 through the earlier of (i) 2001 or (ii) the Base Year.

7 "Royalty Rate" shall mean the royalty rate for a particular crop species and shall be 0.35% where  
9 Licensee's Marketshare is greater than 33.3% and 0.50% where Licensee's Marketshare is less than  
33.3%.

11 "Total Market Size" shall mean the total number of purchased Units for a particular crop species  
13 (i.e., Corn, Sorghum, Soybean, Canola/Rape, Sunflower, etc.) in the Qualifying Region as reported  
in commercially prepared reports, such as Merrits and Doans or any other publication agreed upon  
15 by the parties and listed on Attachment B.3, expressed in Units. In the absence of such reports,  
Licensee shall prepare a report documenting Total Market Size for agreement by the parties.

17 "Unit" shall mean the package size. For the purposes of this Agreement, the following package sizes  
19 shall be used (for crop species not listed below, the Licensee shall provide information on such  
package sizes for agreement by the parties). Package sizes different from below shall be prorated  
21 to the package sizes shown below:

23	Corn:	80,000 kernel package
	Sorghum:	50 pound package
25	Soybean:	50 pound package
	Canola/Rape:	50 pound package
27	Sunflower:	200,000 kernel package

29 For clarity, an example is provided in Attachment B.1. The numbers used in the example are  
examples and should not be considered binding. In the absence of agreement within sixty (60) days  
31 of commencing negotiations hereunder with respect to any issue in this Exhibit B, any such dispute  
shall be resolved pursuant to Section 8.13.2.

1 Attachment B.1

3 Example of Royalty Calculation

5  
7  
9 Assumptions:

11  
13 Crop species: Corn  
Total Market Size: 22,000,000 Units  
15 Licensee Unit Sales: 12,500,000 Units (all units)  
Replant Units: 75,000 (non-royalty bearing)  
17 Sample Units: 200,000 (royalty bearing)  
Full-Price Units: 3,200,000 (royalty bearing)  
19 Base Year: 2003 (first year of Commercial Sale)  
Royalty Base Adjustment Factor (for 1997-2001): 3.79% per year  
21 Single Use Royalty Rate

23 Calculations:

25 Licensee Marketshare:  $12,500,000 \text{ Units} / 22,000,000 \text{ Units} = 56.8\%$   
Royalty Rate: .35%  
27 Qualifying Units: 3,400,000 (sum of Full-Price Units and Sample Units)  
Adjusted Net Royalty Base: See Attachment B.2  
29 Net Sales:  $\$105.91/\text{Units} * 3,400,000 \text{ Units} = \$360,094,000$

31 The royalty due and payable for the year 2003 would be:  
 $\$360,094,000 * .35\% = \$1,260,329$

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Attachment B.2

Example of  
Adjusted Net Royalty Base  
(for Corn)

		Royalty Base Adjustment		Net to Gross	Adjusted Net Royalty Base
	Year	Factor	Royalty Base	Factor	
	1997	N/A	\$ 105.90	20%	\$ 84.72
	1998	3.79%	109.91	20%	87.93
	1999	3.79%	114.08	20%	91.26
	2000	3.79%	118.40	20%	94.72
	2001	3.79%	122.89	20%	98.31
	2002	3.79%	127.55	20%	102.04
	2003	3.79%	132.38	20%	105.91
	2004	3.79%	137.40	20%	109.92
	2005	3.79%	142.61	20%	114.09
	2006	3.79%	148.01	20%	118.41

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Attachment B.3

Commercially Prepared Reports

Merrits and Doans

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1 **EXHIBIT C**

3 **Examples of Calculation of Sublicense Fee**

5 **EXAMPLE 1**

7 Assumption:

9 Value of Licensed Genetic Material = 30

Value of other germ plasm = 70

11 Therefore, percentage of Licensed Genetic Material = 30%

13  
15 Calculation:

17 Factor to apply to License Revenues:  $30\%/2 = 15\%$

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1 **FIRST AMENDED AND RESTATED LICENSE AGREEMENT**

3 THIS LICENSE AGREEMENT (the "Agreement") is made and entered into this <sup>Tu</sup>12 day  
5 of March, 1997 (the "Effective Date") between Pioneer Hi-Bred International, Inc., an Iowa  
7 Company, of 400 Locust Street, Suite 700, Des Moines, IA 50309-2340 ("Licensee"), and  
Kimeragen, Inc., a Delaware corporation, of 300 Pheasant Run, Newtown, PA 18940 ("Licensor").

9 WHEREAS, Licensor represents that it is the exclusive licensee of Thomas Jefferson  
11 University ("TJU") under a U.S. patent and certain U.S. and foreign patent applications with respect  
to a chimeric vector for application in gene therapy developed by TJU and certain methods and  
processes using that chimeric vector; and

13 WHEREAS, Licensee wishes to obtain a non-exclusive, worldwide license under such  
15 technology to conduct research with respect to Plants (as defined below) and to use, sell and license  
Licensed Products (as defined below).

17 NOW THEREFORE, in consideration of the mutual promises and covenants set forth herein  
19 and for good and valuable consideration, the adequacy and sufficiency of which is hereby  
acknowledged, the parties hereby agree as follows:

21 **ARTICLE 1 - DEFINITIONS**

23 1.1 "Affiliate" shall mean any person, which directly or indirectly controls, or is under  
25 common control with, or is controlled by, Licensee. "Control" shall mean the power to direct or  
27 cause the direction of the management and policies of a person, whether through the ownership of  
voting securities by contract or otherwise. "Subsidiary" shall mean with respect to a person, another  
29 person owned as to at least fifty percent (50%) of its equity or other ownership interests; and  
controlled, by the first person.

31 1.2 "Agent" shall mean any company or entity through which Licensee and/or any  
33 Affiliate and/or any Third Party Licensee produces and/or markets Licensed Products on behalf of  
such person.

35 1.3 "Chimera" shall mean any synthetic oligonucleotide of DNA and RNA and/or  
37 derivatives intended to create a specific alteration in a target sequence of the genome of a cell, the  
manufacture, use or sale of which falls within the disclosure and/or claims of U.S. patent no.  
5,565,350 or any application or patent claiming the same priority date as that patent.

39 1.4 "Commercial Sale" shall mean a sale of any Licensed Product by Licensee or any  
41 Affiliate, Agent or Third Party Licensee to an end user customer of that Licensed Product.

1.5 "Confidential Information" shall mean all information, in whatever form, which is disclosed by either party to the other prior to or subsequent to the Effective Date of this Agreement.

1.6 "Enabling Technology" shall mean, Intellectual Property which covers the manufacture, design, delivery and/or use of Chimera, molecules, compounds, drugs, adjuvants, proteins or other material that are used in conjunction with Chimera to alter, modify or deliver DNA and/or RNA, in each case to the extent that they facilitate: (i) dissolution or and/or suspension of the Chimera (and associated agents); (ii) transit of Chimera across cell walls and membranes (including, without limitation, cellular, nuclear, mitochondrial and chloroplast membranes); (iii) protection of Chimera from degradation or inactivation; (iv) target localization to, and accurate pairing of, a target with Chimera; (v) enzyme localization to a Chimera target pairing site; (vi) using or working in conjunction with Chimera to make accurate base changes in genomic and/or target sequences as predicated in any given Chimera design; and any other Intellectual Property which covers aspects of the Chimera design, manufacture, storage, use, mixture, and/or manipulation and/or that improves and/or changes in any way characteristics of such Chimera to locate the desired target and attract or operate on or with appropriate cellular components and/or functions to perform efficient and specific base changes (including without limitation insertions or deletions); but shall not include any such Intellectual Property which is specific to the DNA and/or RNA sequence being altered, modified or delivered.

1.7 "Field" means Licensed Products that are Plants, but excludes Pharmaceutical Use. For clarification, the Field includes development of Plants using Licensed Products that are Plant cell protoplasts.

1.8 "Full-Price Units" shall mean Units of Licensed Product that are invoiced to end user customers and are not Sample Units or Replant Units.

1.9 "Intellectual Property" shall mean without limitation, all patents and patent applications (including utility patents and plant patents and applications for utility patents and plant patents), patentable inventions, plant variety certificates and applications, know-how, trade secrets, techniques and ideas and all technical documentation and/or information which further includes DNA and/or RNA sequences and/or modifications thereof and genes, oligos and proteins, and associated methods and all applications therefor.

1.10 "Kimeragen Licensors" shall mean TJU and any other person that licenses any part of the Licensors Technology to Licensor, including licensors of Third Party Enabling Technology.

1.11 "License Revenues" shall mean all revenues received from Third Party Licensees including, but not limited to, license fees, royalties, and milestone payments.

1.12 "Licensed Genetic Material" shall mean genetic material that has been altered, identified, modified or created (in that generation or an earlier generation) by the use (in whole or in part) of the Licensor Technology which is the subject of any Patent Right or of any other Licensor Technology which Licensee would not have the right to use but for this License.

1.13 "Licensed Products" shall mean products containing Licensed Genetic Material.

1.14 "Licensee Enabling Technology" shall mean all Enabling Technology owned by or licensed to Licensee or any Affiliate.

1.15 "Licensee Product Rights" shall mean any patent or patent application (including any utility patent or plant patent and any application for a utility patent or plant patent), Plant Variety Certificate or plant variety application owned or licensed by Licensee or any Affiliate which covers any Licensed Product or the sale, use, development or production of any Licensed Products.

1.16 "Licensor Technology" shall mean all Enabling Technology owned by or licensed to Licensor, including Third Party Enabling Technology. Licensor Technology shall further include all Patent Rights that cover Licensor Technology.

1.17 "Patent Rights" shall mean patents and patent applications listed in Exhibit A and all foreign counterparts thereof in the Territory, including all divisionals and continuations, continuations in part, additions, confirmations, renewals, extensions, reexaminations and reissues of patents and patent applications and their equivalents covering the Licensor Technology. Patents and patent applications and all foreign counterparts thereof in the Territory covering Third Party Enabling Technology, including all divisionals and continuations, continuations in part, additions, confirmations, renewals, extensions, reexaminations and reissues of patents and patent applications and their equivalents shall be deemed to be included in Exhibit A and to be part of the Patent Rights upon (i) becoming Third Party Enabling Technology and (ii) being licensed to Licensee hereunder.

1.18 "Patent Term" shall mean the period from the Effective Date until the expiration of the last to expire of any Patent Right having a valid claim which, but for this Agreement, Licensee would infringe by any activity permitted under this Agreement.

1.19 "Plants" shall mean multicellular rooted organisms containing chlorophyll and cellulose cell walls.

1.20 "Pharmaceutical Use" shall mean (a) manufacture, synthesis and/or metabolism in Plants (naturally or through genetic engineering) of compounds, precursors or other products with pharmaceutical applications as active ingredients; (b) all harvesting, extraction, refinement, production and sale of such pharmaceutical compounds, precursors or other products; and (c) all uses related to such pharmaceutical compounds, precursors or other products that are or would be

regulated by the FDA Centers of Biological Evaluation and Research (CBER), Drug Evaluation and Research (CDER), Devices and Radiologic Health (CDRH) and Veterinary Medicine (CVM) in any species, and all foreign agencies regulating similar subject matter.

1.21 "Replant Units" shall mean Units of Licensed Product that are invoiced to end user customers at a reduced value for use to replace defective seed and/or seed loss due to flooding or other damage or quality problems.

1.22 "Sample Units" shall mean Units of Licensed Product that are invoiced at no value to end user customers for promotional purposes.

1.23 "Territory" shall mean the world.

1.24 "Third Party Enabling Technology" shall mean Enabling Technology owned or controlled by other licensees of the Licensor Technology that has been licensed to Licensor.

1.25 "Third Party Licensee" shall mean any company or entity, other than an Agent, that is licensed by Licensee and/or any Affiliate to Licensed Products or Licensed Genetic Material.

1.26 "Unit" shall have the meaning specified on Exhibit B.

1.27 "University" shall mean a university or other institution of higher education that is exempt from taxation under clause 501(a) of the Code (26 U.S.C. § 501(a)).

## ARTICLE 2--LICENSE

2.1 Grant of License. Licensor hereby grants Licensee, upon the terms and conditions set forth in this Agreement, a royalty-bearing non-exclusive license under the Licensor Technology in the Territory to use Licensor Technology to conduct product development in the Field and to make, use, sell, import and export Licensed Products in the Field.

2.1.1 Licensee shall have the right to subcontract to Licensee's Subsidiaries and to Universities activities relating to the development, and to Licensee's Affiliates and Agents activities relating to the growing and marketing of Licensed Products, for the purposes of conducting Licensee's ongoing business. Such subcontracting shall not be considered a sublicense, and Licensee shall be responsible for such activities as if they were the activities of Licensee. Without limiting the foregoing, Licensee shall not subcontract any development work to any Subsidiary or University without requiring that the results of such development, if Enabling Technology, be subject to the license provided in Section 2.3.1.

1           2.1.2 Licensee shall have the right to sublicense its rights hereunder for activities  
3 within the Field to Third Party Licensees but only to the following extent:

5           (a) Licensee shall have the right to sublicense Licensed Genetic Material  
7 alone;

9           (b) Licensee shall have the right to sublicense Licensed Genetic Material  
11 incorporated into other germ plasm; and

13           (c) Licensee shall have the right to sublicense the growing and marketing  
15 of Licensed Products.

17           2.1.3 Licensee shall have the right to assign in part the license granted in Section  
19 2.1 to Pioneer Overseas Corporation ("POC") within the Field; provided that POC shall not  
21 further assign any rights under this Agreement other than together with, and not separate  
23 from, any other permitted assignment by Licensee in accordance with Section 8.4; and  
25 provided further that Licensee gives Licensor prompt written notice of the exercise of that  
27 right and the scope of the grant. Notwithstanding any exercise by Licensee of rights under  
29 this Section 2.1.3, Licensee shall be responsible for activities of POC as if they were  
activities by Licensee.

31           2.1.4 No license is granted hereunder for sale of Licensed Products as commodity  
33 grain, except to the extent that seed grain is discarded as a result of obsolescence or quality  
35 defects.

37           2.2 Improvements. During the term of this Agreement, the parties shall promptly disclose  
39 to each other in reasonable detail, including available written protocols of the methodology and of  
41 the materials used, and any available replicated data obtained, any newly developed or controlled  
Licensee Enabling Technology or Licensor Technology.

### 31           2.3 Further Licenses.

33           2.3.1 Licensee hereby grants to Licensor a worldwide license (substantially on the  
35 terms contained in this present license, but excluding payment obligations other than Section  
37 3.8, and recognizing the respective roles of the parties to the license back) limited solely to  
39 applications in conjunction with Chimera, to Licensee Enabling Technology on a non-  
41 exclusive basis for all applications with the right to grant sublicenses (which may further  
include the right to grant sublicenses). Licensor shall sublicense Licensee Enabling  
Technology of a specific type to each particular licensee only to the extent (i) such licensee  
has the similar obligation to grant Licensor a license (including the right to grant sublicenses)  
under its rights in that particular type of Enabling Technology; (ii) Licensee is granted rights

1 to such Enabling Technology as part of the Licensor Technology; and (iii) such licensee has  
2 covenanted substantially as provided in Section 2.3.2. Without limiting Section 2.3.2, such  
3 license shall not grant any rights to Intellectual Property owned or licensed by Licensee or  
4 any Affiliate prior to the Effective Date.

5 2.3.2 Licensee covenants for the benefit of Licensor and Licensor's other licensees  
6 of Enabling Technology in the Field (who may enforce this provision directly) not to assert  
7 any patent owned or controlled by Licensee to prevent use of any Enabling Technology in  
8 conjunction with Chimera pursuant to a license from Licensor in the Field.

9 2.3.3 In negotiating with a third party any potential license for Enabling  
10 Technology from that third party that is exclusive or would otherwise prevent that third party  
11 or Licensee from granting rights to Licensor or other licensees of Enabling Technology,  
12 Licensee shall either (i) obtain the right to sublicense such Enabling Technology to Licensor  
13 and its other licensees of Enabling Technology, or (ii) include Licensor in such negotiations  
14 so that Licensor may seek to obtain rights for use with Chimera.

15 2.4 Protection of Technology. Licensee shall not use any Licensor Technology, nor shall  
16 Licensor use any Licensee Enabling Technology, for any purpose other than as provided in this  
17 Agreement, unless such technology shall come into the public domain.

18 2.5 Acknowledgment of Rights. Licensee acknowledges that Licensee's right to use the  
19 Licensor Technology arises only out of the licenses granted under this Agreement. All Licensed  
20 Products shall bear a patent notice on the label to the extent, if any, as may be required under the  
21 laws of the country in Territory in which the Licensed Products are sold.

## 22 ARTICLE 3-PAYMENT

23 3.1 Payment. In consideration of the rights granted herein, Licensee shall pay to  
24 Licensor:

25 3.1.1 A one-time non-refundable payment of one hundred thousand dollars  
26 (US\$100,000) upon the date of execution of this Agreement. This payment shall be  
27 attributable in part to issued patents as set forth in Exhibit A (ninety thousand dollars  
28 (US\$90,000)) and in part to know-how, and rights to continuations of such patents; and  
29 rights in future patents and other technology as set forth in Exhibit A and elsewhere in this  
30 Agreement (ten thousand dollars (US\$10,000));

1           3.1.2 A one-time non-refundable payment of nine hundred thousand (US\$900,000)  
for the conduct of research on behalf of Licensee in pursuit of Milestone IA (as provided in  
3   Section 3.1.3 below);

5           3.1.3 Further one-time non-refundable payments of: (i) five hundred thousand  
dollars (US\$500,000) for the conduct of research on behalf of Licensee in pursuit of  
7   Milestone II upon the achievement of Milestone IA (as provided below); (ii) five hundred  
thousand dollars (US\$500,000) for the conduct of research on behalf of Licensee in pursuit  
9   of Milestone II upon the achievement of Milestone IB (as provided below); and (iii) one  
million dollars (US\$1,000,000) as a bonus for the successful completion of Milestone II. All  
11 payments will be payable within thirty (30) days of the earlier of (a) achievement by  
Licensee of each milestone; or (b) written notification to Licensee by Licensor of the  
13 achievement of each Milestone, together with written protocols of the methodology and  
materials used, and data which has been replicated at least once demonstrating that such  
15 Milestone was reached;

17           (a) Milestone IA: proof of principle of Licensor Technology in a dicot  
plant species as demonstrated by a genomic sequence altered as predicated by a  
19 specific Chimera design;

21           (b) Milestone IB: proof of principle of Licensor Technology in a  
monocot plant species as demonstrated by a genomic sequence altered as predicated  
23 by a specific Chimera design; and

25           (c) Milestone II: creation of any Plant using or incorporating (in any  
generation) Licensor Technology and/or Licensee Enabling Technology as  
27 demonstrated by the trait of the target altered as predicated by a specific Chimera  
design and such trait is transmitted to at least its next generation progeny;

29           provided that if any such milestone is reached prior to Licensee executing this Agreement,  
31 the payment due for such milestone under this Section 3.1.3 shall be added to the up-front  
fee payable under Sections 3.1.1 and 3.1.2;

33           3.1.4 A further non-refundable payment of one million dollars (US\$1,000,000)  
35 upon the earlier of (i) filing of the first patent application (including any application for a  
utility patent or plant patent) or plant variety protection application in the Territory with  
37 respect to any Licensed Products by Licensee or any Affiliate, or designating Licensee or any  
Affiliate as assignee; or (ii) the first Commercial Sale;

39           3.1.5 A royalty per Unit of Licensed Products as specified in Exhibit B for use of  
41 Licensor Technology in developing Licensed Products, which the parties agree for



1 convenience shall be structured as set out in Section 3.2. Notwithstanding anything to the  
3 contrary herein, in a series of transactions, only the Commercial Sale shall be treated as  
royalty bearing for the purpose of this Agreement; and

5 3.1.6 Royalties as provided in Section 3.4.

7 3.2 Royalty Conditions. Royalties shall:

9 3.2.1 be payable for the duration of the Patent Term:

11 (a) on Commercial Sales by Licensee and/or Affiliates and/or Agents of  
13 Licensed Products that are Full-Price Units or Sample Units, and

15 (b) uses of Licensed Products by Licensee and/or any Affiliate and/or any  
Agent unless the result of that use is a subsequent royalty-bearing sale under Section  
17 3.2.1(a), with royalty to be payable on the Licensed Products used in the same amount as if  
such Licensed Products had been the subject of a Commercial Sale of Full-Price Units

19 in both cases where such Licensed Products are either:

21 (i) at the time and place of development, covered by a pending or issued  
claim in the Patent Rights; or

23 (ii) at the time and place of either use, production or sale, covered by a  
25 pending or issued claim in the Patent Rights;

27 (for clarification, it is agreed that a Licensed Product shall be covered by a pending or issued  
claim in the Patent Rights at the time and place of development where the process of  
29 development and/or products used in that development is or are covered by such a claim);

31 3.2.2 be payable for a period of five (5) years after the Patent Term (not as post-  
expiration royalties but for use of Licensor Technology during the Patent Term to develop  
33 Licensed Products, the payment for which has been agreed for convenience shall be  
measured by sales of Licensed Products), on Commercial Sales by Licensee and/or Affiliates  
35 and/or Agents of those Licensed Products described in Section 3.2.1(i) which are Full-Price  
Units or Sample Units and uses under Section 3.2.1(b) of those Licensed Products described  
37 in Section 3.2.1(i); provided that, at the time of production, sale or use, the Licensed Product  
or its production, sale or use, is covered by a Licensee Product Right;

39 3.2.3 accrue upon the sale, as determined by U.S. GAAP, of Licensed Products by  
41 Licensee and/or Affiliates and/or Agents or upon use pursuant to Section 3.2.1(b);

1           3.2.4 be due and payable within sixty (60) days of the end of each fiscal year of the  
Licensee in U.S. dollars. Subject to the next sentence, any and all withholding taxes levied  
3 on account of royalties or milestones accruing under this Article 3 shall be paid by Licensee,  
on behalf of Licensor. If laws or regulations require withholding of said taxes on royalties,  
5 such taxes may be deducted from such remittable royalty only if (i) such taxes will be paid  
by Licensee, in the name of Licensor to the proper taxing authority, and (ii) proof of payment  
7 and any other documentation required by Licensor to obtain credit for any such payment  
from the U.S. tax authorities shall be sent to Licensor no later than forty-five (45) days  
9 following December 31st of each reporting year. Taxes may not be withheld from the  
amount of milestone payments made to Licensor.

11           3.3 At any time after the first anniversary of the Effective Date, but not more frequently  
13 than once in any twenty-four (24) month period, Licensee may give written notice to Licensor that  
it desires to negotiate a lump sum payment in lieu of royalties payable under Sections 3.1.5 and 3.4  
15 for any one or more Licensed Products. Within thirty (30) days of receiving such notice, Licensor  
shall commence negotiations with Licensee to determine an appropriate amount for such lump sum  
17 payment for each Licensed Product nominated by Licensee. If the parties are unable to agree on an  
amount for each Licensed Product nominated by Licensee within sixty (60) days of the  
19 commencement of such negotiations, Licensor may notify Licensee that it will not accept a lump  
sum payment in lieu of royalties at that time, at all, or for those Licensed Products with respect to  
21 which no agreement was reached.

#### 23           3.4 License Revenues.

25           3.4.1 Licensor shall be entitled to share in all License Revenues derived by  
Licensee and/or any Affiliate. Where License Revenues are payable to Licensee in cash or  
27 equivalent legal tender pursuant to Section 2.1.2(a), Licensee shall pay Licensor fifty per  
cent (50%) of such License Revenues within thirty (30) days after the calendar quarter in  
29 which such License Revenues are received.

31           3.4.2 In the event that Licensee proposes to:

33           (a) barter or trade a sublicense under Section 2.1.2 (including any license  
to an Agent for purposes other than producing and/or marketing any Licensed  
35 Product for or on behalf of Licensee and/or any Affiliate) in return for other  
technology, or Licensee shall otherwise receive non-cash consideration for such  
37 sublicense (in whole or in part); and/or

39           (b) sublicense pursuant to Sections 2.1.2(b) or (c),

1 then Licensee shall not proceed with such sublicense without Licensors consent, which shall  
3 be predicated upon agreement between the parties on an appropriate fee payable by Licensee  
5 to Licensor with respect to such license. Such fee shall be equal to the proportional value  
7 of the Licensed Genetic Material as compared to any other germ plasm licensed, expressed  
9 as a percentage and divided by two (2), provided that in no event shall the amount of royalty  
11 payable to Licensor be less than that amount which would be payable pursuant to Section  
13 3.1.5 on the sale or use of the Licensed Products sold or used by the Third Party Licensee.  
15 An example of such a calculation is provided on Exhibit C. Such percentage shall be applied  
to the value of any such barter or trade plus any cash or cash equivalent forming part of the  
consideration, payable within sixty (60) days of the earlier of entry into such license or  
conclusion of the procedure of Section 8.13.2, or with respect to ongoing payments, within  
sixty (60) days of receipt of payment by Licensee. In the event that the parties are unable to  
agree on an appropriate fee within sixty (60) days of Licensee notifying Licensor of the  
proposed license, Licensee may enter into the license subject to the procedure of  
Section 8.13.2 to determine the fee payable to Licensor.

17 3.4.3 Licensee shall be responsible for monitoring and reporting on the sales of  
19 Affiliates, Third Party Licensees and Agents to the extent information is necessary for the  
calculation of royalties, and shall be responsible for all royalties due to Licensor.

21 3.5 Reports. Licensee shall provide royalty reports to Licensor together with each  
23 payment made under Sections 3.1.5 and 3.4. Such reports shall contain all information relating to  
Licensee's sales as well as sales by Affiliates, Third Party Licensees and Agents and License  
Revenues received, as reasonably necessary to verify amounts due under this Agreement, including:

25 (a) a listing of all Licensed Products qualifying for royalties sold or used by  
27 Licensee, Affiliates, Third Party Licensees and Agents, sorted by individual product, crops,  
species, country and number of Units;

29 (b) all License Revenues received, sorted by Third Party Licensee, license fees,  
31 royalties and milestone and other payments;

33 (c) royalty calculation; and

35 (d) royalties due and payable.

37 3.6 Records. Licensee shall also keep full and accurate records at its principal place of  
39 business in the United States or, if Licensee has no principal place of business in the United States,  
at a location agreed upon in writing by the parties, of all Licensed Products developed, used, grown,  
distributed, or sold by Licensee, Affiliates, Third Party Licensees and Agents and any other records  
41 reasonably necessary to enable verification of reports provided under Section 3.5.

1           3.7 Audit. Records kept by Licensee pursuant to Section 3.6 shall be open, at reasonable  
3 times during business hours, to an inspection by a mutually agreed upon Certified Public  
5 Accountant, at Licensor's expense, for the purposes of verifying Licensee's royalty payments under  
7 this Agreement. If a shortage of greater than five percent (5%) is established in any payment due  
9 hereunder, Licensee shall reimburse Licensor for the cost of such inspection and promptly pay such  
11 overdue amount together with interest at the rate specified in Section 3.9. In addition, in the event  
that Licensee or a ~~Subsidiary~~ <sup>an affiliate</sup> has commercialized a Licensed Product but Licensee has failed to  
make any royalty payments hereunder with respect to such Licensed Product, then Licensee shall  
promptly pay all such past due royalties plus interest of twenty-five percent (25%) per annum,  
calculated from the date such royalties became due until the date such royalties are paid. *ESM*  
*ofc*

13           3.8 Third Party Royalties. In any case where use of the Licensor Technology is in the  
15 future subject to a royalty (whether lump-sum or payable by reference to sales) to a third party (other  
17 than a royalty to TJU with respect to Licensor Technology licensed by Licensor as at the date  
19 hereof), then Licensee shall, in addition to the royalty specified in Section 3.1, be responsible for  
21 payment to Licensor of a further amount equal to the royalty payable to the third party with respect  
23 to Licensee's use under this Agreement, except to the extent Licensee shall be separately licensed  
25 by that third party with respect to such use. In any case where use of the Licensee Enabling  
Technology is in the future subject to a royalty (whether lump-sum or payable by reference to sales)  
to a third party, Licensor shall be responsible for payment to Licensee of an amount equal to the  
royalty payable to the third party with respect to Licensor's or its licensees use of such Enabling  
Technology under this Agreement, except to the extent Licensor or any of its licensees shall be  
separately licensed by that third party with respect to such use.

27           3.9 Late Payments. All payments to be made by the Licensee to the Licensor hereunder  
29 shall bear interest at the prime or equivalent rate as quoted by Citibank N.A., New York, New York,  
on the day the payment is overdue plus two percent (2%) per annum from the date payment becomes  
overdue, until paid.

31           3.10 Royalty Structure. Licensee represents and warrants that: (i) it currently markets its  
33 seed products through a system pursuant to which all seed produced by Licensee, Affiliates and  
35 Agents is invoiced by Licensee and sold to the end user customer (i.e., the grower of commodity  
37 grain); (ii) seeds are either sold as Full-Price Units, Sample Units or Replant Units and not in any  
39 other manner; (iii) seeds are not sold as foundation stock other than to Affiliates or Agents where  
41 seed produced is either a royalty-bearing use or Commercial Sale under this Agreement; and (iv)  
seeds are not sold for purposes other than growing commodity grain except as provided in Section  
2.1.4. The parties acknowledge that it is the intention of this Agreement that Licensor receive  
royalties and payments based on Licensee's business as presently structured of the amounts set forth  
herein. In the event the structure of Licensee's business changes such that Licensee receives  
revenues in a different manner, the parties shall restructure the royalty and payment mechanisms set

1 forth herein to achieve equivalent royalty and payment amounts using, if applicable, a royalty  
2 structure offered to Licensee pursuant to Section 7.6. In the absence of agreement within sixty (60)  
3 days of commencing negotiations hereunder, any such dispute shall be resolved pursuant to Section  
4 8.13.2.

5 3.11 Effect on Agreement. This Agreement shall not become effective until payments as  
6 described in Sections 3.1.1 and 3.1.2 have been made.

#### 7 ARTICLE 4--PATENT INFRINGEMENT

8  
9 Licensee shall notify Licensor promptly of any action, claim or threat of patent infringement suit,  
10 either oral or written, or the commencement of any such patent infringement suit against Licensee  
11 relating to the Licensor Technology. The parties shall cooperate in the development and execution  
12 of a strategy to defend against any such action against Licensee, Licensor or other licensee of  
13 Licensed Technology in the Field by a third party. Each party shall also notify the other promptly  
14 of any infringement, in the Field, of the Patent Rights by a third party of which that party becomes  
15 aware. In the event of a suspected infringement of the Patent Rights by a third party with respect to  
16 Licensed Products being developed or commercialized by Licensee, both parties will consult with  
17 each other and any other affected licensees of Licensor to determine the appropriate strategy to  
18 attempt to prevent such infringement.  
19

#### 20 ARTICLE 5--WARRANTIES & INDEMNITIES

##### 21 5.1 Warranties.

22 5.1.1 Licensor represents and warrants that it has full right, power and authority to  
23 enter into this Agreement and that the terms of this Agreement do not conflict with any other  
24 contractual obligations it has.

25 5.1.2 Licensor represents and warrants that, as of the execution date, it knows of  
26 no Intellectual Property that would prevent Licensee from practicing the whole of the  
27 Licensor Technology as described in U.S. Patent No. 5,565,350. Licensor is aware of  
28 numerous patents covering particular nucleic acids, nucleotides, intermediates, gene  
29 fragments and other aspects of genetic technology.

30 5.1.3 Licensor represents and warrants that it has the freedom to enter into this  
31 Agreement and the right to provide the rights and license contemplated in Section 2.1.  
32

5.1.4 Licensors represents and warrants that, as of the Effective Date, Licensors has disclosed to Licensee all known, and knows of no other, royalty obligations pursuant to Section 3.8.

5.2 No Other Warranties. Neither party makes any warranty or representation with respect to the Enabling Technology, nor is either party in any way responsible for the utility of any Enabling Technology. BOTH PARTIES HEREBY EXPRESSLY DISCLAIM ANY AND ALL WARRANTIES AND REPRESENTATIONS, EXPRESS OR IMPLIED, ARISING BY LAW OR CUSTOM, WITH RESPECT TO THE ENABLING TECHNOLOGY, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, PATENTABILITY OR NON-INFRINGEMENT. NEITHER PARTY IN ANY WAY PROMISES THAT THE ENABLING TECHNOLOGY SHALL PRODUCE ANY PARTICULAR RESULTS, PRODUCTS OR PROFITABILITY.

5.3 Indemnities. Licensee hereby waives any claim against Licensor and the Kimeragen Licensors and agrees to indemnify, defend, and hold harmless Licensor and the Kimeragen Licensors and their respective directors, officers, employees and agents, and in the case of the Kimeragen Licensors, their directors, trustees, officers, agents and employees and those of any associated University, from all liabilities, demands, damages, expenses and losses (including without limitation for death, personal injury, illness or property damage, and including reasonable attorneys' fees) arising out of or in connection with Licensee's or its transferee's actions under this Agreement (collectively, the "Indemnified Losses"), including without limitation Indemnified Losses resulting from any exercise or use by Licensee or its transferees of Patent Rights, and any use, sale, or other disposition of Licensed Products by Licensee or its transferees and any claim that Licensee's use, sale, or other disposition of Licensed Products infringes or violates any patent or other Intellectual Property rights. The indemnification rights contained herein are in addition to all rights which Licensor and/or the Kimeragen Licensors may have at law or in equity. Licensee hereby agrees that the Kimeragen Licensors are entitled to enforce this Section 5.3 directly against Licensee. As used in this Section 5.3, Licensee includes its Affiliates, Subsidiaries, contractors and sub-contractors. Notwithstanding the foregoing, the indemnities provided by Licensee herein shall not apply with respect to Licensor to the extent the indemnified losses would be or are covered by the foregoing Licensor's warranties.

## ARTICLE 6--TERM

6.1 Term Of Agreement. This Agreement shall become effective upon signature by both parties and payment by Licensee of the sums specified in Sections 3.1.1 and 3.1.2. Subject to Section 6.3 and Article 3, and to earlier termination as provided herein, all rights and obligations hereunder shall expire upon the conclusion of the Patent Term. Subject to Section 6.3, upon expiration of the royalty obligation pursuant to Article 3, Licensee shall have a fully paid up license to all Licensor Technology licensed during the term of this Agreement without further obligation to Licensor.

### 6.2 Early Termination.

6.2.1 Subject to Section 6.3, Licensee shall have the right to terminate this Agreement by at least sixty (60) days written notice to Licensor.

6.2.2 Subject to Section 6.3, either party may terminate this Agreement with immediate effect by written notice to the other party, if the other party commits a breach of any material obligation under this Agreement and fails to remedy such breach within sixty (60) days after notice of such breach.

6.3 Effects of Termination. Termination or expiration of this Agreement shall not affect the continued enforceability of Sections 2.3, 5.3, 7.2, 7.4, 7.5 and 8.13, and Article 3 (with respect to any sale or use of Licensed Products, until the date on which the last of any royalty payments payable under Article 3 have been paid).

## ARTICLE 7--OTHER AGREEMENTS

7.1 Supply of Chimera. Licensee may obtain its supply of Chimera solely from: (i) Licensor under terms to be negotiated between the parties; (ii) vendors that are licensed by Licensor to produce Chimera or (iii) from its own production.

### 7.2 Confidentiality. Each party shall:

(a) maintain the Confidential Information of the other party in confidence and refrain from disclosing any part of such Confidential Information to any person or entity other than to its employees, consultants, subcontractors or sublicensees whose duties or rights justify the need to know such Confidential Information;

(b) not make any use of the Confidential Information other than for the purpose of carrying out duties and obligations and exercising rights under this Agreement;

1 (c) take all reasonable steps to protect the Confidential Information against  
disclosure, misuse, loss and theft, which steps include the execution by all such persons of  
3 written agreements containing obligations of confidentiality, restricted disclosure and limited  
use relative thereto consistent with this Section 7.2 prior to disclosure of Confidential  
5 Information to them; and

7 (d) in the case of Licensor, in the event that a third party wishes to evaluate  
Confidential Information in connection with a proposed business transaction with Licensor,  
9 disclose only Enabling Technology developed by Licensee hereunder to that third party as  
is necessary to conduct such evaluation, provided that prior to disclosure such third party  
11 executes a written agreement prohibiting use of the Confidential Information for any reason  
other than evaluation of such transactions and containing obligations of confidentiality  
13 consistent with this Section 7.2.

15 The provisions of this Section 7.2 shall not apply to any part of the Confidential Information  
disclosed by one party to the other (a) which is agreed in writing by the disclosing party to be  
17 excluded; or (b) which the receiving party can show was known to or developed by it prior to  
Confidential Information first being received by it from, or disclosed to it by, the disclosing party;  
19 or (c) which is public knowledge, or becomes public knowledge in the future, other than through acts  
or omissions of the receiving party in breach of this Agreement; or (d) which is lawfully obtained  
21 by receiving party from sources independent of the disclosing party who have a lawful right to  
possess and disclose such Information; (e) which it is necessary for the receiving party to disclose  
23 in order to comply with any applicable law or if required to do so by order of any court or any other  
judicial or administrative body, provided that prior to making such disclosure the receiving party  
25 gives the disclosing party notice of the requirement of disclosure and the information to be disclosed;  
or (f) which is to be included in any patent application, provided that prior to filing any such patent  
27 application, the party proposing to file such application shall provide a copy to the other party and  
not file such application until the other party shall have consented, such consent not to be  
29 unreasonably withheld or delayed.

31 7.3 Press Releases. Prior to any press release concerning the execution of this  
Agreement, its terms and conditions, or any subsequent event under this Agreement which either  
33 party considers newsworthy, both parties shall agree on the content and timing, such consent not to  
be unreasonably withheld.

35 7.4 Regulatory Approvals. The parties shall provide to one another (at no cost) all  
37 materials, data and information in their possession needed to seek and obtain regulatory approvals  
necessary for the use and sale of Licensed Products. Neither party shall use any regulatory  
39 information and/or packages developed by the other for the benefit of a third party, except that  
Licensor shall be permitted to use such information to establish master files for filing with regulatory  
41 agencies. Licensee shall comply with all regulatory requirements relating to the Licensed Products



1 and shall take all reasonable or required steps to ensure that the Licensed Products are safe and  
lawful.

3           **7.5    Product Liability Insurance.** Licensee shall obtain and maintain commercial general  
5 liability insurance, including commercial liability, product liability and completed operations  
insurance coverage in a minimum amount of five million dollars (\$5,000,000) per loss including  
7 coverage for contractual liability. Licensor and the Kimeragen Licensors that are Universities and  
their respective officers, directors, trustees, members of governing boards and employees will be  
9 named insureds under all such insurance. Such insurance shall also provide that Licensor and the  
Kimeragen Licensors that are Universities be given notice of any modification thereof and at least  
11 ten (10) days prior written notice of cancellation or termination and the reason therefor. A certificate  
of insurance evidencing such coverage will be provided to Licensor and, upon each annual  
13 anniversary of this Agreement, Licensee shall provide written confirmation issued by the insurer or  
an independent insurance agent confirming that insurance is maintained in accordance with the  
15 above requirements. Subject to Licensee obtaining consent from the Kimeragen Licensors that are  
Universities, Licensee may elect to be self-insured in accordance with reasonable business practices,  
17 provided that the above requirements are met.

19           **7.6    Equivalent Royalty Structure.** Licensor undertakes to Licensee that it will not offer  
any third party licensee of Licensor Technology in the Field any up-front payment or milestone  
21 payment lower than those set out in Sections 3.1.1, 3.1.2, 3.1.3 and 3.1.4; or any royalty rate lower  
than that set out in Section 3.1.5 and Exhibit B without offering such terms to Licensee to the extent  
23 applicable to Licensee's business structure.

25           **7.7    Committee.** A committee of not more than six (6) persons comprising an equal  
number of representatives of each party shall be formed (the "Committee") and shall meet not less  
27 than once each calendar year to discuss research, progress toward achievement of the milestones set  
out in Sections 3.1.3 and 3.1.4 and commercialization of Licensed Products. At such meeting,  
29 Licensee shall provide to Licensor, in a document marked "confidential," a description of the  
projects to which Licensee or its Subsidiaries or Universities subcontracted by Licensee have applied  
31 Chimera, a description of Licensed Products developed by Licensee or its Subsidiaries or  
Universities, and a notification of any such Licensed Products that have been commercialized.

33           **7.8    Trademarks and Use of Names.** Neither party shall have any right to use any  
trademark or trade name of the other without the other party's prior written consent, and then subject  
35 to such terms and conditions as may be agreed to in writing by the parties. If Licensee develops a  
Licensed Product for commercialization, the Committee shall consider in good faith whether use of  
37 Licensor's trademarks or a description of Licensor Technology and/or a particular aspect thereof in  
marketing brochures, literature and labeling for any Licensed Product is appropriate and desirable,  
39 and the appropriate payment and other terms for such trademark use. Neither party shall use the  
name of the other, nor shall Licensee use the name of any Kimeragen Licensor, or the names of any  
41

1 of their respective staff members, employees or students or any adaptation thereof in any advertising,  
3 promotional or sales literature to the extent such use might imply a relationship between the parties,  
5 or endorsement by either party or any Kimeragen Licensor of any act or thing or any product or  
method described in such material, without the prior written consent of the other party and the  
Kimeragen Licensor where applicable, which consent shall not be unreasonably withheld.

## 7 ARTICLE 8--MISCELLANEOUS PROVISIONS

9 8.1 Force Majeure. Neither party shall be liable for failure to perform its obligations  
11 (other than any obligation to pay money) hereunder for so long as that failure may be the result of  
13 an event beyond its reasonable control (a "force majeure" event), provided that such party uses all  
reasonable efforts to comply with the terms of this Agreement to the extent that it is able to do so.

15 8.2 Entire Agreement. This Agreement, together with all Exhibits attached hereto,  
17 constitutes the entire Agreement between the parties with respect to the present subject matter, all  
prior negotiations, agreements and understandings being expressly canceled hereby, including that  
License Agreement dated March 10, 1997.

19 8.3 Amendment. This Agreement may be amended only by a written agreement  
21 embodying the full terms of the amendment signed by authorized representatives of both parties.

23 8.4 Assignment. Neither party may assign their rights or obligations under this  
25 Agreement without prior written approval from the other party, except as provided for in Section  
27 2.1.3 and except that neither party shall require the consent of the other for the assignment of this  
Agreement as part of the sale or transfer of substantially all of its business, provided that any  
purchaser of such business (or surviving entity in the case of any merger) expressly agrees to assume  
that party's rights and obligations under this Agreement.

29 8.5 Severability. Should any provision of this Agreement be illegal, invalid or  
31 unenforceable under applicable law, such provision shall be deleted from this Agreement and the  
33 remaining provisions of this Agreement shall be construed as if such illegal, invalid or unenforceable  
provision had not been contained herein and the remainder of this Agreement shall not be affected  
thereby. In addition, the parties agree that by mutual agreement or through judicial modification such  
35 illegal, invalid or unenforceable provision shall be replaced by a provision or new provisions added  
that shall be as similar as possible in economic and business objectives to the provision deleted but  
37 which shall be valid, legal and enforceable.

39 8.6 No Strict Construction. The language used in this Agreement shall be deemed to be  
41 the language chosen by both parties hereto to express their mutual intent and no rule of strict  
construction against either party shall apply to any term or condition of this Agreement.

1       8.7    Relationship of Parties. Nothing contained in this Agreement shall be construed as  
3   creating a partnership, joint venture, agency, franchise or an association of any kind between the  
parties or otherwise.

5       8.8    No Waiver. The failure of one party hereto to enforce at any time any of the  
7   provisions of this Agreement, or any rights in respect thereto, or to exercise any election herein  
provided, shall in no way be considered to be a waiver of such provision, rights or elections or in any  
way to affect the validity of this Agreement. Any waiver must be in writing.

9       8.9    Interpretation. The headings contained in this Agreement are for convenience only  
11   and shall not affect the interpretation of this Agreement. In this Agreement, the word "including"  
shall be deemed to be followed by "without limitation", the words "hereof" and "herein" and  
13   "hereunder" refer to this Agreement as a whole, and the singular includes the plural and vice versa.

15       8.10 Notices. Notices shall be given by first class mail, by Federal Express or other  
17   recognized courier requiring signature on receipt, and shall be addressed to the other party at the  
address set forth below (or at such address as a party may specify by notice to the other):

19               If to Licensee: Pioneer Hi-Bred International Inc.  
                  7300 NW 62nd  
21               P.O. Box 1004  
                  Johnson, IA 50131-1004

23                       Attention: Director, Research Technology Services  
25                       Telephone: (515) 270-3600

27               If to Licensor: Kimeragen, Inc.  
                  300 Pheasant Run  
29               Newtown, PA 18940

31                       Attention: President  
                  Telephone: (215) 504-4444

33       8.11 Governing Law and Jurisdiction. Pursuant to Section 5-1401 of the New York  
35   General Obligations Law, this Agreement shall be governed by and construed in accordance with  
the laws of the State of New York without giving effect to any choice of law or conflict of law  
37   provision or rule that would cause the application of the laws of any jurisdiction other than the State  
of New York.

1        8.12 Counterparts. This Agreement may be executed in one or more counterparts, each  
3 of which shall be deemed an original, but all of which together shall constitute one and the same  
instrument.

5        8.13 Dispute Resolution.

7        8.13.1 The parties shall work together to remedy any difficulties which may arise in  
9 connection with this Agreement. All disputes arising out of this Agreement (other than  
disputes arising under Section 3.4, 3.10 or Exhibit B, which shall be resolved in the manner  
described below in Section 8.13.2) shall be referred to decision forthwith to a senior  
11 executive of each party who is, if possible, not involved in the dispute. If no agreement can  
be reached through this process within thirty (30) days of request by one party to the other  
13 to nominate a senior executive for dispute resolution, then either party hereto shall be entitled  
to bring proceedings relating to such dispute. Any and all such proceedings shall be brought  
15 in a court having jurisdiction over the parties in the County of Manhattan, New York.

17        8.13.2 Any dispute arising under Section 3.4 of this Agreement which is not resolved  
between the parties within sixty (60) days of Licensee notifying Licensor of a proposed  
19 sublicense, or any dispute under Section 3.10 or Exhibit B, shall be referred to a partner of  
Arthur Andersen & Company in Philadelphia nominated by that firm (the "Arbitrator") for  
21 decision on (i) in the case of Section 3.4, which proposal for the fees payable to Licensor for  
such sublicense, (ii) in the case of Section 3.10, which revised payment structure, last put  
23 forward by the respective parties prior to the referral to arbitration is the most reasonable in  
the circumstances; or (iii) in the case of Exhibit B, which proposal last put forward by the  
25 respective parties prior to the referral to arbitration is the most reasonable in the  
circumstances. The Arbitrator shall have no discretion to make any determination other than  
27 a choice between the last proposal put forward by each of the parties. The parties may each  
present written material to the Arbitrator to support their last offer, provided such material  
29 is submitted to the Arbitrator within thirty (30) days of the matter being referred to the  
Arbitrator, and the parties will use their best efforts to ensure that the Arbitrator shall make  
31 a decision in the manner prescribed in this Section and notify the parties of such decision  
within thirty (30) days of the expiration of the time for the parties to submit written material,  
33 or earlier waiver by the parties of that right. The Arbitrator's decision shall be irrevocable  
and fully accepted by the parties, and Licensee shall pay the fee stipulated by the Arbitrator  
35 to Licensor with its next royalty payment after such decision. The party whose proposal was  
not chosen by the Arbitrator shall bear all reasonable costs incurred by both parties incurred  
37 in connection with such arbitration.

39                    \* \* \* \* \*

1 IN WITNESS WHEREOF, the parties have caused their duly authorized representative to  
3 execute this Agreement as of the Effective Date.

5 PIONEER HI-BRED INTERNATIONAL, INC.

7 By:   
9

11 Name: Anthony T. Cavalieri

13 Title: Vice President

15 KIMERAGEN, INC.

17 By:   
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21 Name: Gerald L. Messerschmidt, M.D.

23 Title: President

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**EXHIBIT A**

**Patent Rights<sup>1</sup>**

- United States Patent Number 5,565,350

- Foreign counterparts to '350 patent:

<u>Country</u>	<u>Application Serial No.</u>
Australia	13995/95
Canada	2,178,729
China, People's Republic	94194935.4
European Patent Convention	95905337.2
Korea (South)	703040/96
Japan	7-516367
New Zealand	278490

- Saliwanchik, Lloyd & Saliwanchik docket no. KIM 100-P  
"Alteration of Plant and Yeast Genes"

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<sup>1</sup> Rights of Licensee in above listed patents and applications are limited to Enabling Technology in the Field and specifically exclude any right to practice any gene or sequence specific subject matter of any such patents or applications.

1 **EXHIBIT B**

3 **Royalty Calculations**

5 It is the intent of the Parties to have an equitable and auditable protocol for determining royalties.  
The following definitions shall be used:

7 "Adjusted Net Royalty Base" shall mean the Royalty Base, as annually adjusted by Royalty Base  
9 Adjustment Factor beginning in 1998 and compounded annually, less the Gross-to Net Factor. An  
example of the calculation of the Adjusted Net Royalty Base is set forth in Attachment B.2.

11 "Average Net Published Price" shall mean the average price in the U.S.A. for all non-transgenic  
13 "Performance Elite" products as published in the "Master Card" or equivalent.

15 "Base Year" shall mean the first year a Licensed Product that is a Qualifying Unit is sold in the  
U.S.A.

17 "Gross-to-Net Factor" shall mean the multiplier that is a proxy for the typical and traditional  
19 conversion of list price to invoice price for Full-Price Units (regardless of crop species), which  
equals 20%.

21 "Licensee Marketshare" shall mean Licensee Unit Sales divided by Total Market Size, expressed as  
23 a percentage.

25 "Licensee Unit Sales" shall mean Licensee's Unit sales of a particular crop species in the Qualifying  
Region as reported in commercially prepared reports, such as Merritts and Doans or any other  
27 publication agreed upon by the parties and listed on Attachment B.3, expressed in Units. In the  
absence of such reports, Licensee shall prepare a report documenting Licensee Unit Sales for  
29 agreement by the parties.

31 "Multiple-Use Royalty Rate" shall mean 0.70% or 1.0% for two (2) or more uses of Licensor  
Technology in one version of Licensed Product where Licensee's Marketshare is greater than 33.3%  
33 or less than 33.3%, respectively.

35 "Net Sales" shall mean the Adjusted Net Royalty Base times the number of Qualifying Units.

37 "Qualifying Region" shall mean the region composed of North America, South America and the  
European Union.

39 "Qualifying Units" shall mean Sample Units and Full-Price Units sold or used which create a royalty  
41 obligation pursuant to Section 3.2.

1 "Royalty Base" shall mean the royalty base and shall be one hundred and five dollars and ninety  
cents (US\$105.90) per Unit of Corn for 1997. For other crop species, the Royalty Base shall be  
3 calculated using the same protocol and formula as was used for Corn.

5 "Royalty Base Adjustment Factor" shall mean the average increase in the Average Net Published  
Price over a period starting in 1997 through the earlier of (i) 2001 or (ii) the Base Year.

7 "Royalty Rate" shall mean the royalty rate for a particular crop species and shall be 0.35% where  
9 Licensee's Marketshare is greater than 33.3% and 0.50% where Licensee's Marketshare is less than  
33.3%.

11 "Total Market Size" shall mean the total number of purchased Units for a particular crop species  
13 (i.e., Corn, Sorghum, Soybean, Canola/Rape, Sunflower, etc.) in the Qualifying Region as reported  
in commercially prepared reports, such as Merritts and Doans or any other publication agreed upon  
15 by the parties and listed on Attachment B.3, expressed in Units. In the absence of such reports,  
Licensee shall prepare a report documenting Total Market Size for agreement by the parties.

17 "Unit" shall mean the package size. For the purposes of this Agreement, the following package sizes  
19 shall be used (for crop species not listed below, the Licensee shall provide information on such  
package sizes for agreement by the parties). Package sizes different from below shall be prorated  
21 to the package sizes shown below:

23	Corn:	80,000 kernel package
	Sorghum:	50 pound package
25	Soybean:	50 pound package
	Canola/Rape:	50 pound package
27	Sunflower:	200,000 kernel package

29 For clarity, an example is provided in Attachment B.1. The numbers used in the example are  
examples and should not be considered binding. In the absence of agreement within sixty (60) days  
31 of commencing negotiations hereunder with respect to any issue in this Exhibit B, any such dispute  
shall be resolved pursuant to Section 8.13.2.



Attachment B.1

**Example of Royalty Calculation**

Assumptions:

Crop species: Corn  
Total Market Size: 22,000,000 Units  
Licensee Unit Sales: 12,500,000 Units (all units)  
Replant Units: 75,000 (non-royalty bearing)  
Sample Units: 200,000 (royalty bearing)  
Full-Price Units: 3,200,000 (royalty bearing)  
Base Year: 2003 (first year of Commercial Sale)  
Royalty Base Adjustment Factor (for 1997-2001): 3.79% per year  
Single Use Royalty Rate

Calculations:

Licensee Marketshare:  $12,500,000 \text{ Units} / 22,000,000 \text{ Units} = 56.8\%$   
Royalty Rate: .35%  
Qualifying Units: 3,400,000 (sum of Full-Price Units and Sample Units)  
Adjusted Net Royalty Base: See Attachment B.2  
Net Sales:  $\$105.91/\text{Units} * 3,400,000 \text{ Units} = \$360,094,000$

The royalty due and payable for the year 2003 would be:  
 $\$360,094,000 * .35\% = \$1,260,329$

Attachment B.2

Example of  
Adjusted Net Royalty Base  
(for Corn)

Year	Royalty Base Adjustment Factor	Royalty Base	Net to Gross Factor	Adjusted Net Royalty Base
1997	N/A	\$ 105.90	20%	\$ 84.72
1998	3.79%	109.91	20%	87.93
1999	3.79%	114.08	20%	91.26
2000	3.79%	118.40	20%	94.72
2001	3.79%	122.89	20%	98.31
2002	3.79%	127.55	20%	102.04
2003	3.79%	132.38	20%	105.91
2004	3.79%	137.40	20%	109.92
2005	3.79%	142.61	20%	114.09
2006	3.79%	148.01	20%	118.41

Attachment B.3

Commercially Prepared Reports

Merrits and Doans

000693

**EXHIBIT C**

**Examples of Calculation of Sublicense Fee**

**EXAMPLE 1**

**Assumption:**

Value of Licensed Genetic Material = 30

Value of other germ plasm = 70

Therefore, percentage of Licensed Genetic Material = 30%

**Calculation:**

Factor to apply to License Revenues:  $30\%/2 = 15\%$



1.5 "Confidential Information" shall mean all information, in whatever form, which is disclosed by either party to the other prior to or subsequent to the Effective Date of this Agreement.

1.6 "Enabling Technology" shall mean, Intellectual Property which covers the manufacture, design, delivery and/or use of Chimera, molecules, compounds, drugs, adjuvants, proteins or other material that are used in conjunction with Chimera to alter, modify or deliver DNA and/or RNA, in each case to the extent that they facilitate: (i) dissolution or and/or suspension of the Chimera (and associated agents); (ii) transit of Chimera across cell walls and membranes (including, without limitation, cellular, nuclear, mitochondrial and chloroplast membranes); (iii) protection of Chimera from degradation or inactivation; (iv) target localization to, and accurate pairing of, a target with Chimera; (v) enzyme localization to a Chimera target pairing site; (vi) using or working in conjunction with Chimera to make accurate base changes in genomic and/or target sequences as predicated in any given Chimera design; and any other Intellectual Property which covers aspects of the Chimera design, manufacture, storage, use, mixture, and/or manipulation and/or that improves and/or changes in any way characteristics of such Chimera to locate the desired target and attract or operate on or with appropriate cellular components and/or functions to perform efficient and specific base changes (including without limitation insertions or deletions); and any part of Intellectual Property that covers products (including, without limitation, plants, plant cells and seeds) made by use of Chimera, whether such products are defined as products per se, as products-by-process, as products defined by traits or other characteristics, or however such products are defined; except that Enabling Technology shall not include any part of Intellectual Property that is:

- specific to a gene (including the promoters, enhancers, and other cis-acting control elements of such gene) being altered, modified or delivered or specific to homologs of such gene being altered, modified or delivered; wherein any such gene shall be at least defined by its function (such as function of its expression product, such as RNA or protein) or structure (i.e. partial or complete sequence of its expression product) or shall be defined by some structural characteristics (e.g. partial or complete nucleotide sequence); or
- specific to the sequence of a Chimera that is used; or
- specific to a plant variety, or to a group of closely related plant varieties;

or any Intellectual Property that is a plant patent, a plant variety certificate or patent that is specific to a plant variety or any application therefor.

1.7 "Field" means Licensed Products that are Plants, but excludes Pharmaceutical Use. For clarification, the Field includes development of Plants using Licensed Products that are Plant cell protoplasts.

1.8 "Full-Price Units" shall mean Units of Licensed Product that are invoiced to end user customers and are not Sample Units or Replant Units.

1.9 "Intellectual Property" shall mean without limitation, all patents and patent applications (including utility patents and plant patents and applications for utility patents and plant patents), patentable inventions, plant variety certificates and applications, know-how, trade secrets, techniques and ideas and all technical documentation and/or information which further includes DNA and/or RNA sequences and/or modifications thereof and genes, oligos and proteins, and associated methods and all applications therefor.

1.10 "Kimeragen Licensors" shall mean TJU and any other person that licenses any part of the Licensors Technology to Licensors, including licensors of Third Party Enabling Technology.

1.11 "License Revenues" shall mean all revenues received from Third Party Licensees including, but not limited to, license fees, royalties, and milestone payments.

1.12 "Licensed Genetic Material" shall mean genetic material that has been altered, identified, modified or created (in that generation or an earlier generation) by the use (in whole or in part) of the Licensors Technology which is the subject of any Patent Right or of any other Licensors Technology which Licensee would not have the right to use but for this License.

1.13 "Licensed Products" shall mean products containing Licensed Genetic Material.

1.14 "Licensee Enabling Technology" shall mean all Enabling Technology owned by or licensed to Licensee or any Affiliate.

1.15 "Licensee Product Rights" shall mean any patent or patent application (including any utility patent or plant patent and any application for a utility patent or plant patent), Plant Variety Certificate or plant variety application owned or licensed by Licensee or any Affiliate which covers any Licensed Product or the sale, use, development or production of any Licensed Products.

1.16 "Licensors Technology" shall mean all Enabling Technology owned by or licensed to Licensors, including Third Party Enabling Technology which Licensors is permitted to license pursuant to Section 2.3.1.

1.17 "Patent Rights" shall mean patents and patent applications listed in Exhibit A included in the Enabling Technology and all foreign counterparts thereof in the Territory, as well as all divisionals and continuations, continuations in part, additions, confirmations, renewals, extensions, reexaminations and reissues of such patents and patent applications ("Related Patents") and all future patents and patent applications and all foreign counterparts thereof in the Territory and Related Patents included in the Enabling Technology owned or controlled by Licensors other than Third Party Enabling Technology, all of which shall be deemed to be included in Exhibit A. Patents and patent applications and all foreign counterparts thereof in the Territory included in the Third Party Enabling Technology, including all Related Patents, shall be deemed to be included in Exhibit A and to be part of the Patent

1 Rights upon (i) becoming Third Party Enabling Technology and (ii) being licensed to Licensee  
hereunder.

3  
5 1.18 "Patent Term" shall mean the period from the Effective Date until the expiration of  
the last to expire of any Patent Right having a valid claim which, but for this Agreement, Licensee  
would infringe by any activity permitted under this Agreement.

7  
9 1.19 "Plants" shall mean multicellular rooted organisms containing chlorophyll and  
cellulose cell walls.

11 1.20 "Pharmaceutical Use" shall mean (a) manufacture, synthesis and/or metabolism in  
Plants (naturally or through genetic engineering) of compounds, precursors or other products with  
13 pharmaceutical applications as active ingredients; (b) all harvesting, extraction, refinement,  
production and sale of such pharmaceutical compounds, precursors or other products; and (c) all  
15 uses related to such pharmaceutical compounds, precursors or other products that are or would be  
regulated by the FDA Centers of Biological Evaluation and Research (CBER), Drug Evaluation and  
17 Research (CDER), Devices and Radiologic Health (CDRH) and Veterinary Medicine (CVM) in any  
species, and all foreign agencies regulating similar subject matter. It shall not be a Pharmaceutical  
19 Use to develop a Plant where a product from such Plant shall have a subsequently discovered and  
not anticipated or intended pharmaceutical application.

21  
23 1.21 "Replant Units" shall mean Units of Licensed Product that are invoiced to end user  
customers at a reduced value for use to replace defective seed and/or seed loss due to flooding or  
other damage or quality problems.

25  
27 1.22 "Sample Units" shall mean Units of Licensed Product that are invoiced at no value  
to end user customers for promotional purposes.

29 1.23 "Territory" shall mean the world.

31 1.24 "Third Party Enabling Technology" shall mean Enabling Technology owned or  
controlled by other licensees of the Licensor Technology that has been licensed to Licensor.

33  
35 1.25 "Third Party Licensee" shall mean any company or entity, other than an Agent, that  
is licensed by Licensee and/or any Affiliate to Licensed Products or Licensed Genetic Material.

37 1.26 "Unit" shall have the meaning specified on Exhibit B.

39 1.27 "University" shall mean a university or other institution of higher education that is  
exempt from taxation under clause 501(a) of the Code (26 U.S.C. § 501(a)).



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## ARTICLE 2--LICENSE

2.1 Grant of License. Licensor hereby grants Licensee, upon the terms and conditions set forth in this Agreement, a royalty-bearing non-exclusive license under the Licensor Technology in the Territory to use Licensor Technology to conduct product development in the Field and to make, use, sell, import and export Licensed Products in the Field.

2.1.1 Licensee shall have the right to subcontract to Licensee's Subsidiaries and to Universities activities relating to the development, and to Licensee's Affiliates and Agents activities relating to the growing and marketing of Licensed Products, for the purposes of conducting Licensee's ongoing business. Such subcontracting shall not be considered a sublicense, and Licensee shall be responsible for such activities as if they were the activities of Licensee. Without limiting the foregoing, Licensee shall not subcontract any development work to any Subsidiary or University without requiring that the results of such development, if Enabling Technology, be subject to the license provided in Section 2.3.1.

2.1.2 Licensee shall have the right to sublicense its rights hereunder for activities within the Field to Third Party Licensees but only to the extent necessary to exercise the following rights:

(a) Licensee shall have the right to license Licensed Genetic Material in any form or medium not incorporated into germ plasm;

(b) Licensee shall have the right to license Licensed Genetic Material incorporated into germ plasm; and

(c) Licensee shall have the right to license the breeding, multiplication and marketing of Licensed Products.

2.1.3 Licensee shall have the right to assign in part the license granted in Section 2.1 to Pioneer Overseas Corporation ("POC") within the Field; provided that POC shall not further assign any rights under this Agreement other than together with, and not separate from, any other permitted assignment by Licensee in accordance with Section 8.4; and provided further that Licensee gives Licensor prompt written notice of the exercise of that right and the scope of the grant. Notwithstanding any exercise by Licensee of rights under this Section 2.1.3, Licensee shall be responsible for activities of POC as if they were activities by Licensee.

2.1.4 Licensee shall not itself exercise any right granted hereunder for sale of Licensed Products as commodity grain, except to the extent that seed grain is discarded as a result of obsolescence or quality defects, without limiting Licensee's rights to sell Licensed Products for such purpose or Licensee's rights under Section 2.1.2.

1           2.2    Improvements. During the term of this Agreement, the parties shall disclose to each  
other any newly developed or controlled Licensee Enabling Technology or Licensors Technology  
3    which shall be the subject of a patent application promptly after the first non-provisional filing of a  
patent application covering such technology. The parties shall provide information on such technology  
5    in reasonable detail, including available written protocols of the methodology and of the materials  
used, and any available replicated data obtained.

7  
9           2.3    Further Licenses.

11           2.3.1   Licensee hereby grants to Licensors a worldwide license (substantially on the  
terms contained in this present license, but excluding payment obligations other than Section  
13    3.8, and recognizing the respective roles of the parties to the license back) limited solely to  
applications in conjunction with Chimera, to Licensee Enabling Technology on a non-  
exclusive basis for all applications with the right to grant sublicenses (which may further  
15    include the right to grant sublicenses). Licensors shall sublicense Licensee Enabling  
Technology of a specific type to each particular licensee of the Licensors Technology in the  
17    Field only to the extent (i) such licensee has the similar obligation to grant Licensors a license  
(including the right to grant sublicenses) under its rights in that particular type of Enabling  
19    Technology; (ii) Licensee is granted rights to such other licensee Enabling Technology as  
part of the Licensors Technology; and (iii) such licensee has covenanted substantially as  
21    provided in Section 2.3.2. Without limiting Section 2.3.2, such license shall not grant any  
rights to Intellectual Property owned or licensed by Licensee or any Affiliate prior to the  
23    Effective Date.

25           2.3.2   Licensee covenants for the benefit of Licensors and Licensors's other licensees  
of Enabling Technology in the Field (who may enforce this provision directly) not to assert any  
27    claim to Enabling Technology of a patent owned or controlled by Licensee against the use in  
the Field of any Enabling Technology in conjunction with Chimera pursuant to a license from  
29    Licensors ("Licensed Use in the Field") nor to assert infringement of any such patent claim by  
the manufacture, sale or use of a Licensed Product, if the assertion of infringement is based on  
31    Licensed Use in the Field or the result of Licensed Use in the Field. This covenant applies to  
Licensee Enabling Technology owned or controlled by Licensee both prior to and after the  
33    Effective Date.

35           2.3.3   In negotiating with a third party any potential license for Enabling  
Technology from that third party that is exclusive or would otherwise prevent that third party  
37    or Licensee from granting rights to Licensors or other licensees of Enabling Technology,  
Licensee shall either (i) obtain the right to sublicense such Enabling Technology to Licensors  
39    and its other licensees of Enabling Technology, or (ii) include Licensors in such negotiations  
so that Licensors may seek to obtain rights for use with Chimera.

2.4 Protection of Technology. Licensee shall not use any Licensor Technology, nor shall Licensor use any Licensee Enabling Technology, for any purpose other than as provided in this Agreement, unless such technology shall come into the public domain.

2.5 Acknowledgment of Rights. Licensee acknowledges that Licensee's right to use the Licensor Technology arises only out of the licenses granted under this Agreement. All Licensed Products shall bear a patent notice on the label to the extent, if any, as may be required under the laws of the country in Territory in which the Licensed Products are sold.

### ARTICLE 3-PAYMENT

3.1 Payment. In consideration of the rights granted herein, Licensee shall pay to Licensor:

3.1.1 A one-time non-refundable payment of one hundred thousand dollars (US\$100,000) upon the Effective Date of this Agreement. This payment shall be attributable in part to issued patents as set forth in Exhibit A (ninety thousand dollars (US\$90,000)) and in part to know-how, and rights to continuations of such patents; and rights in future patents and other technology as set forth in Exhibit A and elsewhere in this Agreement (ten thousand dollars (US\$10,000));

3.1.2 A one-time non-refundable payment of nine hundred thousand (US\$900,000) for the conduct of research on behalf of Licensee in pursuit of Milestone IA (as provided in Section 3.1.3 below);

3.1.3 Further one-time non-refundable payments of: (i) five hundred thousand dollars (US\$500,000) for the conduct of research on behalf of Licensee in pursuit of Milestone II upon the achievement of Milestone IA (as provided below); (ii) five hundred thousand dollars (US\$500,000) for the conduct of research on behalf of Licensee in pursuit of Milestone II upon the achievement of Milestone IB (as provided below); and (iii) one million dollars (US\$1,000,000) as a bonus for the successful completion of Milestone II. All payments will be payable within thirty (30) days of the earlier of (a) achievement by Licensee of each Milestone; or (b) written notification to Licensee by Licensor of the achievement of each Milestone, together with written protocols of the methodology and materials used, and data which has been replicated at least once demonstrating that such Milestone was reached;

(a) Milestone IA: proof of principle of Licensors Technology in a dicot plant species as demonstrated by a genomic sequence altered as predicated by a specific Chimera design;

1 (b) Milestone IB: proof of principle of Licensors Technology in a  
3 monocot plant species as demonstrated by a genomic sequence altered as predicated  
by a specific Chimera design; and

5 (c) Milestone II: creation of any Plant using or incorporating (in any  
7 generation) Licensors Technology and/or Licensee Enabling Technology as  
demonstrated by the trait of the target altered as predicated by a specific Chimera  
9 design and such trait is transmitted to at least its next generation progeny;

11 provided that if any Milestone is reached prior to Licensee executing this Agreement, the  
payment due for such Milestone under this Section 3.1.3 shall be added to the up-front fee  
13 payable under Sections 3.1.1 and 3.1.2;

15 3.1.4 A further non-refundable payment of one million dollars (US\$1,000,000)  
upon the earlier of (i) filing of the first patent application (including any application for a  
17 utility patent or plant patent) or plant variety protection application in the Territory with  
respect to any Licensed Products by Licensee or any Affiliate, or designating Licensee or  
any Affiliate as assignee; or (ii) the first Commercial Sale;

19 3.1.5 A royalty per Unit of Licensed Products as specified in Exhibit B for use of  
21 Licensors Technology in developing Licensed Products, which the parties agree for  
convenience shall be structured as set out in Section 3.2. Notwithstanding anything to the  
23 contrary herein, in a series of transactions, only the Commercial Sale shall be treated as  
royalty bearing for the purpose of this Agreement; and

25 3.1.6 Royalties as provided in Section 3.4.

27 3.2 Royalty Conditions. Royalties shall:

29 3.2.1 be payable for the duration of the Patent Term:

31 (a) on Commercial Sales by Licensee and/or Affiliates and/or Agents of  
33 Licensed Products that are Full-Price Units or Sample Units, and

35 (b) uses of Licensed Products by Licensee and/or any Affiliate and/or any  
Agent unless the result of that use is a subsequent royalty-bearing sale under Section  
37 3.2.1(a), with royalty to be payable on the Licensed Products used in the same amount as if  
such Licensed Products had been the subject of a Commercial Sale of Full-Price Units

39 in both cases where such Licensed Products are either:  
41

1 (i) at the time and place of development, covered by a pending or issued  
claim in the Patent Rights; or

3 (ii) at the time and place of either use, production or sale, covered by a  
5 pending or issued claim in the Patent Rights;

7 (for clarification, it is agreed that a Licensed Product shall be covered by a pending or issued  
claim in the Patent Rights at the time and place of development where the process of  
9 development and/or products used in that development is or are covered by such a claim);

11 3.2.2 be payable for a period of five (5) years after the Patent Term (not as post-  
expiration royalties but for use of Licensors Technology during the Patent Term to develop  
13 Licensed Products, the payment for which has been agreed for convenience shall be  
measured by sales of Licensed Products), on Commercial Sales by Licensee and/or Affiliates  
15 and/or Agents of those Licensed Products described in Section 3.2.1(i) which are Full-Price  
Units or Sample Units and uses under Section 3.2.1(b) of those Licensed Products described  
17 in Section 3.2.1(i); provided that, at the time of production, sale or use, the Licensed Product  
or its production, sale or use, is covered by a Licensee Product Right;

19 3.2.3 accrue upon the sale, as determined by U.S. GAAP, of Licensed Products by  
Licensee and/or Affiliates and/or Agents or upon use pursuant to Section 3.2.1(b);

23 3.2.4 be due and payable within sixty (60) days of the end of each fiscal year of the  
Licensee in U.S. dollars. Subject to the next sentence, any and all withholding taxes levied  
25 on account of royalties or milestones accruing under this Article 3 shall be paid by Licensee,  
on behalf of Licensors. If laws or regulations require withholding of said taxes on royalties,  
27 such taxes may be deducted from such remittable royalty only if (i) such taxes will be paid  
by Licensee, in the name of Licensors to the proper taxing authority, and (ii) proof of  
29 payment and any other documentation required by Licensors to obtain credit for any such  
payment from the U.S. tax authorities shall be sent to Licensors no later than forty-five (45)  
31 days following December 31st of each reporting year. Taxes may not be withheld from the  
amount of milestone payments made to Licensors.

33 3.3 At any time after the first anniversary of the Effective Date, but not more frequently  
35 than once in any twenty-four (24) month period, Licensee may give written notice to Licensors that  
it desires to negotiate a lump sum payment in lieu of royalties payable under Sections 3.1.5 and 3.4  
37 for any one or more Licensed Products. Within thirty (30) days of receiving such notice, Licensors  
shall commence negotiations with Licensee to determine an appropriate amount for such lump sum  
39 payment for each Licensed Product nominated by Licensee. If the parties are unable to agree on an  
amount for each Licensed Product nominated by Licensee within sixty (60) days of the  
41 commencement of such negotiations, Licensors may notify Licensee that it will not accept a lump

1 sum payment in lieu of royalties at that time, at all, or for those Licensed Products with respect to  
3 which no agreement was reached.

### 5 3.4 License Revenues.

7 3.4.1 Licensors shall be entitled to share in all License Revenues derived by  
9 Licensee and/or any Affiliate. Where License Revenues are payable to Licensee in cash or  
11 equivalent legal tender for rights to Licensed Genetic Material not incorporated into germ  
13 plasm, Licensee shall pay Licensors fifty per cent (50%) of such License Revenues within  
15 thirty (30) days after the calendar quarter in which such License Revenues are received.

17 3.4.2 In the event that Licensee proposes to:

19 (a) barter or trade rights to Licensed Genetic Material in any form or  
21 medium not incorporated into germ plasm, or to Licensed Genetic Material  
23 incorporated into germ plasm, or to Licensed Products (including any license to an  
25 Agent for purposes other than producing and/or marketing any Licensed Product for  
27 or on behalf of Licensee and/or any Affiliate), in return for other technology, or  
29 Licensee shall otherwise receive non-cash consideration for such rights (in whole or  
31 in part); and/or

33 (b) grant rights to Licensed Genetic Material incorporated into germ plasm  
35 or rights to Licensed Products,

37 then Licensee shall not proceed with such license without Licensors consent, which shall  
39 be predicated upon agreement between the parties on an appropriate fee payable by Licensee  
to Licensors with respect to such license. Such fee shall be equal to the proportional value  
of the Licensed Genetic Material as compared to any other germ plasm licensed, expressed  
as a percentage and divided by two (2), provided that in no event shall the amount of royalty  
payable to Licensors be less than that amount which would be payable pursuant to Section  
3.1.5 on the sale or use of the Licensed Products sold or used by the Third Party Licensee.  
An example of such a calculation is provided on Exhibit C. Such percentage shall be applied  
to the value of any such barter or trade plus any cash or cash equivalent forming part of the  
consideration, payable within sixty (60) days of the earlier of entry into such license or  
conclusion of the procedure of Section 8.13.2, or with respect to ongoing payments, within  
sixty (60) days of receipt of payment by Licensee. In the event that the parties are unable  
to agree on an appropriate fee within sixty (60) days of Licensee notifying Licensors of the  
proposed license, Licensee may enter into the, license subject to the procedure of  
Section 8.13.2 to determine the fee payable to Licensors.

1           3.4.3 Licensee shall be responsible for monitoring and reporting on the sales of  
3           Affiliates, Third Party Licensees and Agents to the extent information is necessary for the  
            calculation of royalties, and shall be responsible for all royalties due to Licensor.

5           3.5 Reports. Licensee shall provide royalty reports to Licensor together with each  
7           payment made under Sections 3.1.5 and 3.4. Such reports shall contain all information relating to  
            Licensee's sales as well as sales by Affiliates, Third Party Licensees and Agents and License  
            Revenues received, as reasonably necessary to verify amounts due under this Agreement, including:

9                   (a) a listing of all Licensed Products qualifying for royalties sold or used by  
11                  Licensee, Affiliates, Third Party Licensees and Agents, sorted by individual product, crops,  
                    species, country and number of Units;

13                   (b) all License Revenues received, sorted by Third Party Licensee, license fees,  
15                  royalties and milestone and other payments;

17                   (c) royalty calculation; and

19                   (d) royalties due and payable.

21           3.6 Records. Licensee shall also keep full and accurate records at its principal place of  
23           business in the United States or, if Licensee has no principal place of business in the United States,  
            at a location agreed upon in writing by the parties, of all Licensed Products developed, used, grown,  
25           distributed, or sold by Licensee, Affiliates, Third Party Licensees and Agents and any other records  
            reasonably necessary to enable verification of reports provided under Section 3.5.

27           3.7 Audit. Records kept by Licensee pursuant to Section 3.6 shall be open, at reasonable  
29           times during business hours, to an inspection by a mutually agreed upon Certified Public  
            Accountant, at Licensor's expense, for the purposes of verifying Licensee's royalty payments under  
31           this Agreement. If a shortage of greater than five percent (5%) is established in any payment due  
            hereunder, Licensee shall reimburse Licensor for the cost of such inspection and promptly pay such  
33           overdue amount together with interest at the rate specified in Section 3.9. In addition, in the event  
            that Licensee or an Affiliate has commercialized a Licensed Product but Licensee has failed to make  
            any royalty payments hereunder with respect to such Licensed Product, then Licensee shall promptly  
35           pay all such past due royalties plus interest of twenty-five percent (25%) per annum, calculated from  
            the date such royalties became due until the date such royalties are paid.

37           3.8 Third Party Royalties. In any case where use of the Licensor Technology is in the  
39           future subject to a royalty (whether lump-sum or payable by reference to sales) to a third party (other  
            than a royalty to TJU with respect to Licensor Technology licensed by Licensor as at the date  
41           hereof), then Licensee shall, in addition to the royalty specified in Section 3.1, be responsible for  
            payment to Licensor of a further amount equal to the royalty payable to the third party with respect

1 to Licensee's use under this Agreement, except to the extent Licensee shall be separately licensed  
3 by that third party with respect to such use. In any case where use of the Licensee Enabling  
5 Technology is in the future subject to a royalty (whether lump-sum or payable by reference to sales)  
7 to a third party, Licensor shall be responsible for payment to Licensee of an amount equal to the  
royalty payable to the third party with respect to Licensor's or its licensees use of such Enabling  
Technology under this Agreement, except to the extent Licensor or any of its licensees shall be  
separately licensed by that third party with respect to such use.

9 3.9 Late Payments. All payments to be made by the Licensee to the Licensor hereunder  
11 shall bear interest at the prime or equivalent rate as quoted by Citibank N.A., New York, New York,  
on the day the payment is overdue plus two percent (2%) per annum from the date payment becomes  
overdue, until paid.

13 3.10 Royalty Structure. Licensee represents and warrants that: (i) it currently markets its  
15 seed products through a system pursuant to which all seed produced by Licensee, Affiliates and  
Agents is invoiced by Licensee and sold to the end user customer (i.e., the grower of commodity  
17 grain); (ii) seeds are either sold as Full-Price Units, Sample Units or Replant Units and not in any  
other manner; (iii) seeds are not sold as foundation stock other than to Affiliates or Agents where  
19 seed produced is either a royalty-bearing use or Commercial Sale under this Agreement; and (iv)  
seeds are not sold for purposes other than growing commodity grain except as provided in Section  
21 2.1.4. The parties acknowledge that it is the intention of this Agreement that Licensor receive  
royalties and payments based on Licensee's business as presently structured of the amounts set forth  
23 herein. In the event the structure of Licensee's business changes such that Licensee receives  
revenues in a different manner, the parties shall restructure the royalty and payment mechanisms set  
25 forth herein to achieve equivalent royalty and payment amounts using, if applicable, a royalty  
structure offered to Licensee pursuant to Section 7.6. In the absence of agreement within sixty (60)  
27 days of commencing negotiations hereunder, any such dispute shall be resolved pursuant to Section  
8.13.2.

29 3.11 Effect on Agreement. This Agreement shall not become effective until payments as  
31 described in Sections 3.1.1 and 3.1.2 have been made.

#### 33 ARTICLE 4-PATENT INFRINGEMENT

35 Licensee shall notify Licensor promptly of any action, claim or threat of patent infringement suit,  
37 either oral or written, or the commencement of any such patent infringement suit against Licensee  
relating to the Licensor Technology. The parties shall cooperate in the development and execution  
39 of a strategy to defend against any such action against Licensee, Licensor or other licensee of  
Licensed Technology in the Field by a third party. Each party shall also notify the other promptly  
41 of any infringement, in the Field, of the Patent Rights by a third party of which that party becomes  
aware. In the event of a suspected infringement of the Patent Rights by a third party with respect to



1 Licensed Products being developed or commercialized by Licensee, both parties will consult with  
3 each other and any other affected licensees of Licensor to determine the appropriate strategy to  
attempt to prevent such infringement.

## 5 ARTICLE 5--WARRANTIES & INDEMNITIES

### 7 5.1 Warranties.

9 5.1.1 Licensor represents and warrants that it has full right, power and authority to  
11 enter into this Agreement and that the terms of this Agreement do not conflict with any other  
contractual obligations it has.

13 5.1.2 Licensor represents and warrants that, as of the execution date, it knows of  
15 no Intellectual Property that would prevent Licensee from practicing the whole of the  
Licensor Technology as described in U.S. Patent No. 5,565,350. Licensor is aware of  
17 numerous patents covering particular nucleic acids, nucleotides, intermediates, gene  
fragments and other aspects of genetic technology.

19 5.1.3 Licensor represents and warrants that it has the freedom to enter into this  
Agreement and the right to provide the rights and license contemplated in Section 2.1.

21 5.1.4 Licensor represents and warrants that, as of the Effective Date, Licensor has  
23 disclosed to Licensee all known, and knows of no other, royalty obligations pursuant to  
Section 3.8.

25 5.2 No Other Warranties. Neither party makes any warranty or representation with  
27 respect to the Enabling Technology, nor is either party in any way responsible for the utility of any  
Enabling Technology. BOTH PARTIES HEREBY EXPRESSLY DISCLAIM ANY AND ALL  
29 WARRANTIES AND REPRESENTATIONS, EXPRESS OR IMPLIED, ARISING BY LAW OR  
CUSTOM, WITH RESPECT TO THE ENABLING TECHNOLOGY, INCLUDING, WITHOUT  
31 LIMITATION, WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR  
PURPOSE, PATENTABILITY OR NON-INFRINGEMENT. NEITHER PARTY IN ANY WAY  
33 PROMISES THAT THE ENABLING TECHNOLOGY SHALL PRODUCE ANY PARTICULAR  
RESULTS, PRODUCTS OR PROFITABILITY.

35 5.3 Indemnities. Licensee hereby waives any claim against Licensor and the Kimeragen  
37 Licensors and agrees to indemnify, defend, and hold harmless Licensor and the Kimeragen  
Licensors and their respective directors, officers, employees and agents, and in the case of the  
39 Kimeragen Licensors, their directors, trustees, officers, agents and employees and those of any  
associated University, from all liabilities, demands, damages, expenses and losses (including  
41 without limitation for death, personal injury, illness or property damage, and including reasonable  
attorneys' fees) arising out of or in connection with Licensee's or its transferee's actions under this

1 Agreement (collectively, the "Indemnified Losses"), including without limitation Indemnified  
2 Losses resulting from any exercise or use by Licensee or its transferees of Patent Rights, and any  
3 use, sale, or other disposition of Licensed Products by Licensee or its transferees and any claim that  
4 Licensee's use, sale, or other disposition of Licensed Products infringes or violates any patent or  
5 other Intellectual Property rights. The indemnification rights contained herein are in addition to all  
6 rights which Licensor and/or the Kimeragen Licensors may have at law or in equity. Licensee  
7 hereby agrees that the Kimeragen Licensors are entitled to enforce this Section 5.3 directly against  
8 Licensee. As used in this Section 5.3, Licensee includes its Affiliates, Subsidiaries, contractors and  
9 sub-contractors. Notwithstanding the foregoing, the indemnities provided by Licensee herein shall  
10 not apply with respect to Licensor to the extent the indemnified losses would be or are covered by  
11 the foregoing Licensor's warranties.

## 13 ARTICLE 6--TERM

15 6.1 Term Of Agreement. This Agreement shall become effective upon signature by both  
16 parties and payment by Licensee of the sums specified in Sections 3.1.1 and 3.1.2. Subject to  
17 Section 6.3 and Article 3, and to earlier termination as provided herein, all rights and obligations  
18 hereunder shall expire upon the conclusion of the Patent Term. Subject to Section 6.3, upon  
19 expiration of the royalty obligation pursuant to Article 3, Licensee shall have a fully paid up license  
20 to all Licensor Technology licensed during the term of this Agreement without further obligation  
21 to Licensor.

### 23 6.2 Early Termination.

25 6.2.1 Subject to Section 6.3, Licensee shall have the right to terminate this  
26 Agreement by at least sixty (60) days written notice to Licensor.

28 6.2.2 Subject to Section 6.3, either party may terminate this Agreement with  
29 immediate effect by written notice to the other party, if the other party commits a breach of  
30 any material obligation under this Agreement and fails to remedy such breach within sixty  
31 (60) days after notice of such breach.

33 6.3 Effects of Termination. Termination or expiration of this Agreement shall not affect  
34 the continued enforceability of Sections 2.3, 5.3, 7.2, 7.4, 7.5 and 8.13, and Article 3 (with respect  
35 to any sale or use of Licensed Products, until the date on which the last of any royalty payments  
36 payable under Article 3 have been paid).

## ARTICLE 7--OTHER AGREEMENTS

7.1 Supply of Chimera. Licensee may obtain its supply of Chimera solely from:  
(i) Licensor under terms to be negotiated between the parties; (ii) vendors that are licensed by  
Licensor to produce Chimera or (iii) from its own production.

7.2 Confidentiality. Each party shall:

(a) maintain the Confidential Information of the other party in confidence and refrain from disclosing any part of such Confidential Information to any person or entity other than to its employees, consultants, subcontractors or sublicensees whose duties or rights justify the need to know such Confidential Information;

(b) not make any use of the Confidential Information other than for the purpose of carrying out duties and obligations and exercising rights under this Agreement;

(c) take all reasonable steps to protect the Confidential Information against disclosure, misuse, loss and theft, which steps include the execution by all such persons of written agreements containing obligations of confidentiality, restricted disclosure and limited use relative thereto consistent with this Section 7.2 prior to disclosure of Confidential Information to them; and

(d) in the case of Licensor, in the event that a third party wishes to evaluate Confidential Information in connection with a proposed business transaction with Licensor, disclose only Enabling Technology developed by Licensee hereunder to that third party as is necessary to conduct such evaluation, provided that prior to disclosure such third party executes a written agreement prohibiting use of the Confidential Information for any reason other than evaluation of such transactions and containing obligations of confidentiality consistent with this Section 7.2.

The provisions of this Section 7.2 shall not apply to any part of the Confidential Information disclosed by one party to the other (a) which is agreed in writing by the disclosing party to be excluded; or (b) which the receiving party can show was known to or developed by it prior to Confidential Information first being received by it from, or disclosed to it by, the disclosing party; or (c) which is public knowledge, or becomes public knowledge in the future, other than through acts or omissions of the receiving party in breach of this Agreement; or (d) which is lawfully obtained by receiving party from sources independent of the disclosing party who have a lawful right to possess and disclose such Information; (e) which it is necessary for the receiving party to disclose in order to comply with any applicable law or if required to do so by order of any court or any other judicial or administrative body, provided that prior to making such disclosure the receiving party gives the disclosing party notice of the requirement of disclosure and the information to be disclosed; or (f) which is to be included in any patent application, provided that prior to filing any

1 such patent application, the party proposing to file such application shall provide a copy to the other  
2 party and not file such application until the other party shall have consented, such consent not to be  
3 unreasonably withheld or delayed.

5 7.3 Press Releases. Prior to any press release concerning the execution of this  
6 Agreement, its terms and conditions, or any subsequent event under this Agreement which either  
7 party considers newsworthy, both parties shall agree on the content and timing, such consent not to  
8 be unreasonably withheld.

9 7.4 Regulatory Approvals. The parties shall provide to one another (at no cost) all  
10 materials, data and information in their possession needed to seek and obtain regulatory approvals  
11 necessary for the use and sale of Licensed Products. Neither party shall use any regulatory  
12 information and/or packages developed by the other for the benefit of a third party, except that  
13 Licensor shall be permitted to use such information to establish master files for filing with regulatory  
14 agencies. Licensee shall comply with all regulatory requirements relating to the Licensed Products  
15 and shall take all reasonable or required steps to ensure that the Licensed Products are safe and  
16 lawful.

17 7.5 Product Liability Insurance. Licensee shall obtain and maintain commercial general  
18 liability insurance, including commercial liability, product liability and completed operations  
19 insurance coverage in a minimum amount of five million dollars (\$5,000,000) per loss including  
20 coverage for contractual liability. Licensor and the Kimeragen Licensors that are Universities and  
21 their respective officers, directors, trustees, members of governing boards and employees will be  
22 named insureds under all such insurance. Such insurance shall also provide that Licensor and the  
23 Kimeragen Licensors that are Universities be given notice of any modification thereof and at least  
24 ten (10) days prior written notice of cancellation or termination and the reason therefor. A  
25 certificate of insurance evidencing such coverage will be provided to Licensor and, upon each  
26 annual anniversary of this Agreement, Licensee shall provide written confirmation issued by the  
27 insurer or an independent insurance agent confirming that insurance is maintained in accordance  
28 with the above requirements. Subject to Licensee obtaining consent from the Kimeragen Licensors  
29 that are Universities, Licensee may elect to be self-insured in accordance with reasonable business  
30 practices, provided that the above requirements are met.

31 7.6 Equivalent Royalty Structure. Licensor undertakes to Licensee that it will not offer  
32 any third party licensee of Licensor Technology in the Field any up-front payment or milestone  
33 payment lower than those set out in Sections 3.1.1, 3.1.2, 3.1.3 and 3.1.4; or any royalty rate lower  
34 than that set out in Section 3.1.5 and Exhibit B without offering such terms to Licensee to the extent  
35 applicable to Licensee's business structure.

36 7.7 Committee. A committee of not more than six (6) persons comprising an equal  
37 number of representatives of each party shall be formed (the "Committee") and shall meet not less  
38 than once each calendar year to discuss research, progress toward achievement of the milestones set  
39

1 out in Sections 3.1.3 and 3.1.4 and commercialization of Licensed Products. At such meeting,  
3 Licensee shall provide to Licensors, in a document marked "confidential," a description of the  
5 projects to which Licensee or its Subsidiaries or Universities subcontracted by Licensee have  
applied Chimera, a description of Licensed Products developed by Licensee or its Subsidiaries or  
Universities, and a notification of any such Licensed Products that have been commercialized.

7 7.8 Trademarks and Use of Names. Neither party shall have any right to use any  
9 trademark or trade name of the other without the other party's prior written consent, and then subject  
11 to such terms and conditions as may be agreed to in writing by the parties. If Licensee develops a  
13 Licensed Product for commercialization, the Committee shall consider in good faith whether use of  
15 Licensors' trademarks or a description of Licensors' Technology and/or a particular aspect thereof in  
17 marketing brochures, literature and labeling for any Licensed Product is appropriate and desirable,  
19 and the appropriate payment and other terms for such trademark use. Neither party shall use the  
name of the other, nor shall Licensee use the name of any Kimeragen Licensors, or the names of any  
of their respective staff members, employees or students or any adaptation thereof in any  
advertising, promotional or sales literature to the extent such use might imply a relationship between  
the parties, or endorsement by either party or any Kimeragen Licensors of any act or thing or any  
product or method described in such material, without the prior written consent of the other party  
and the Kimeragen Licensors where applicable, which consent shall not be unreasonably withheld.

## ARTICLE 8-MISCELLANEOUS PROVISIONS

23 8.1 Force Majeure. Neither party shall be liable for failure to perform its obligations  
25 (other than any obligation to pay money) hereunder for so long as that failure may be the result of  
27 an event beyond its reasonable control (a "force majeure" event), provided that such party uses all  
reasonable efforts to comply with the terms of this Agreement to the extent that it is able to do so.

29 8.2 Entire Agreement. This Agreement, together with all Exhibits attached hereto,  
31 constitutes the entire Agreement between the parties with respect to the present subject matter, all  
prior negotiations, agreements and understandings being expressly canceled hereby, including that  
License Agreement dated March 10, 1997.

33 8.3 Amendment. This Agreement may be amended only by a written agreement  
35 embodying the full terms of the amendment signed by authorized representatives of both parties.

37 8.4 Assignment. Neither party may assign their rights or obligations under this  
39 Agreement without prior written approval from the other party, except as provided for in Section  
41 2.1.3 and except that neither party shall require the consent of the other for the assignment of this  
Agreement as part of the sale or transfer of substantially all of its business provided that any  
purchaser of such business (or surviving entity in the case of any merger) expressly agrees to assume  
that party's rights and obligations under this Agreement.

1           8.5    Severability. Should any provision of this Agreement be illegal, invalid or  
3   unenforceable under applicable law, such provision shall be deleted from this Agreement and the  
5   remaining provisions of this Agreement shall be construed as if such illegal, invalid or unenforceable  
7   provision had not been contained herein and the remainder of this Agreement shall not be affected  
9   thereby. In addition, the parties agree that by mutual agreement or through judicial modification  
such illegal, invalid or unenforceable provision shall be replaced by a provision or new provisions  
added that shall be as similar as possible in economic and business objectives to the provision  
deleted but which shall be valid, legal and enforceable.

11           8.6    No Strict Construction. The language used in this Agreement shall be deemed to be  
13   the language chosen by both parties hereto to express their mutual intent and no rule of strict  
construction against either party shall apply to any term or condition of this Agreement.

15           8.7    Relationship of Parties. Nothing contained in this Agreement shall be construed as  
17   creating a partnership, joint venture, agency, franchise or an association of any kind between the  
parties or otherwise.

19           8.8    No Waiver. The failure of one party hereto to enforce at any time any of the  
21   provisions of this Agreement, or any rights in respect thereto, or to exercise any election herein  
provided, shall in no way be considered to be a waiver of such provision, rights or elections or in  
any way to affect the validity of this Agreement. Any waiver must be in writing.

23           8.9    Interpretation. The headings contained in this Agreement are for convenience only  
25   and shall not affect the interpretation of this Agreement. In this Agreement, the word "including"  
shall be deemed to be followed by "without limitation", the words "hereof" and "herein" and  
"hereunder" refer to this Agreement as a whole, and the singular includes the plural and vice versa.

27           8.10   Notices. Notices shall be given by first class mail, by Federal Express or other  
29   recognized courier requiring signature on receipt, and shall be addressed to the other party at the  
address set forth below (or at such address as a party may specify by notice to the other):

1 If to Licensee: Pioneer Hi-Bred International Inc.  
3 7300 NW 62nd  
5 P.O. Box 1004  
7 Johnson, IA 50131-1004

9 Attention: Director, Research Technology Services  
11 Telephone: (515) 270-3600

13 If to Licensor: Kimeragen, Inc.  
15 300 Pheasant Run  
17 Newtown, PA 18940

19 Attention: President  
21 Telephone: (215) 504-4444

23 8.11 Governing Law and Jurisdiction. Pursuant to Section 5-1401 of the New York  
25 General Obligations Law, this Agreement shall be governed by and construed in accordance with  
27 the laws of the State of New York without giving effect to any choice of law or conflict of law  
29 provision or rule that would cause the application of the laws of any jurisdiction other than the State  
31 of New York.

33 8.12 Counterparts. This Agreement may be executed in one or more counterparts, each  
35 of which shall be deemed an original, but all of which together shall constitute one and the same  
37 instrument.

39 8.13 Dispute Resolution.

41 8.13.1 The parties shall work together to remedy any difficulties which may arise  
in connection with this Agreement. All disputes arising out of this Agreement (other than  
disputes arising under Section 3.4, 3.10 or Exhibit B, which shall be resolved in the manner  
described below in Section 8.13.2) shall be referred to decision forthwith to a senior  
executive of each party who is, if possible, not involved in the dispute. If no agreement can  
be reached through this process within thirty (30) days of request by one party to the other  
to nominate a senior executive for dispute resolution, then either party hereto shall be  
entitled to bring proceedings relating to such dispute. Any and all such proceedings shall  
be brought in a court having jurisdiction over the parties in the County of Manhattan, New  
York.

8.13.2 Any dispute arising under Section 3.4 of this Agreement which is not resolved  
between the parties within sixty (60) days of Licensee notifying Licensor of a proposed  
sublicense, or any dispute under Section 3.10 or Exhibit B, shall be referred to a partner of

1 Arthur Andersen & Company in Philadelphia or another Certified Public Accountant  
nominated by that firm (the "Arbitrator") for decision on (i) in the case of Section 3.4, which  
3 proposal for the fees payable to Licensor for such license last put forward by the respective  
parties prior to the referral for arbitration is the most reasonable in the circumstances, (ii)  
5 in the case of Section 3.10, which revised payment structure, last put forward by the  
respective parties prior to the referral to arbitration is the most reasonable in the  
7 circumstances; or (iii) in the case of Exhibit B, which proposal last put forward by the  
respective parties prior to the referral to arbitration is the most reasonable in the  
9 circumstances. The Arbitrator shall have no discretion to make any determination other than  
a choice between the last proposal put forward by each of the parties. The parties may each  
11 present written material to the Arbitrator to support their last offer, provided such material  
is submitted to the Arbitrator within thirty (30) days of the matter being referred to the  
13 Arbitrator, and the parties will use their best efforts to ensure that the Arbitrator shall make  
a decision in the manner prescribed in this Section and notify the parties of such decision  
15 within thirty (30) days of the expiration of the time for the parties to submit written material,  
or earlier waiver by the parties of that right. The Arbitrator's decision shall be irrevocable  
17 and fully accepted by the parties, and Licensee shall pay the fee stipulated by the Arbitrator  
to Licensor with its next royalty payment after such decision. The party whose proposal was  
19 not chosen by the Arbitrator shall bear all reasonable costs incurred by both parties incurred  
in connection with such arbitration.

\* \* \* \* \*



1 IN WITNESS WHEREOF, the parties have caused their duly authorized representative to  
3 execute this Agreement as of the Effective Date.

5 PIONEER HI-BRED INTERNATIONAL, INC.

7  
9 By: 

11 Name: Anthony J. Cavalieri

13 Title: Vice President

15 KIMERAGEN, INC.

17  
19 By: 

21 Name: Gerald L. Messerschmidt, M.D.

23 Title: President

1 **EXHIBIT A**

3 **Patent Rights**<sup>1</sup>

- 5
- 7 • United States Patent Number 5,565,350
- 9 • United States Patent application Number 08/709,982 (CON of '350)
- 11 • United States Patent application Number 08/664,487 (P&E Docket No. 7991-013)
- 13 • Foreign counterparts to '350 patent:

15

<u>Country</u>	<u>Application Serial No.</u>
17 Australia	13995/95
Canada	2,178,729
19 China, People's Republic	94194935.4
European Patent Convention	95905337.2
Korea (South)	703040/96
Japan	7-516367
23 New Zealand	278490

- 25 • provisional application Serial No. 60/039,572, entitled "Alteration of Plant and Yeast Genes"
- 27
- 29 • provisional application Serial No. to be assigned entitled "Methods of Introducing Specific Genetic Alterations in Plants" P&E Docket No. 7991-023-888
- 31

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<sup>1</sup> Rights of Licensee in above listed patents and applications are limited to Enabling Technology in the Field and specifically exclude any right to practice any gene or sequence specific subject matter of any such patents or applications.

1 **EXHIBIT B**

3 **Royalty Calculations**

5 It is the intent of the Parties to have an equitable and auditable protocol for determining royalties.  
The following definitions shall be used:

7 "Adjusted Net Royalty Base" shall mean the Royalty Base, as annually adjusted by Royalty Base  
9 Adjustment Factor beginning in 1998 and compounded annually, less the Gross-to Net Factor. An  
example of the calculation of the Adjusted Net Royalty Base is set forth in Attachment B.2.

11 "Average Net Published Price" shall mean the average price in the U.S.A. for all non-transgenic  
13 "Performance Elite" products as published in the "Master Card" or equivalent.

15 "Base Year" shall mean the first year a Licensed Product that is a Qualifying Unit is sold in the  
U.S.A.

17 "Gross-to-Net Factor" shall mean the multiplier that is a proxy for the typical and traditional  
19 conversion of list price to invoice price for Full-Price Units (regardless of crop species), which  
equals 20%.

21 "Licensee Marketshare" shall mean Licensee Unit Sales divided by Total Market Size, expressed  
23 as a percentage.

25 "Licensee Unit Sales" shall mean Licensee's Unit sales of a particular crop species in the Qualifying  
Region as reported in commercially prepared reports, such as Merrits and Doans or any other  
27 publication agreed upon by the parties and listed on Attachment B.3, expressed in Units. In the  
absence of such reports, Licensee shall prepare a report documenting Licensee Unit Sales for  
29 agreement by the parties.

31 "Multiple-Use Royalty Rate" shall mean 0.70% or 1.0% for two (2) or more uses of Licensor  
Technology in one version of Licensed Product where Licensee's Marketshare is greater than 33.3%  
33 or less than 33.3%, respectively.

35 "Net Sales" shall mean the Adjusted Net Royalty Base times the number of Qualifying Units.

37 "Qualifying Region" shall mean the region composed of North America, South America and the  
European Union.

39 "Qualifying Units" shall mean Sample Units and Full-Price Units sold or used which create a royalty  
41 obligation pursuant to Section 3.2.

1 "Royalty Base" shall mean the royalty base and shall be one hundred and five dollars and ninety  
cents (US\$105.90) per Unit of Corn for 1997. For other crop species, the Royalty Base shall be  
3 calculated using the same protocol and formula as was used for Corn.

5 "Royalty Base Adjustment Factor" shall mean the average increase in the Average Net Published  
Price over a period starting in 1997 through the earlier of (i) 2001 or (ii) the Base Year.

7 "Royalty Rate" shall mean the royalty rate for a particular crop species and shall be 0.35% where  
9 Licensee's Marketshare is greater than 33.3% and 0.50% where Licensee's Marketshare is less than  
33.3%.

11 "Total Market Size" shall mean the total number of purchased Units for a particular crop species  
13 (i.e., Corn, Sorghum, Soybean, Canola/Rape, Sunflower, etc.) in the Qualifying Region as reported  
in commercially prepared reports, such as Merritts and Doans or any other publication agreed upon  
15 by the parties and listed on Attachment B.3, expressed in Units. In the absence of such reports,  
Licensee shall prepare a report documenting Total Market Size for agreement by the parties.

17 "Unit" shall mean the package size. For the purposes of this Agreement, the following package sizes  
19 shall be used (for crop species not listed below, the Licensee shall provide information on such  
package sizes for agreement by the parties). Package sizes different from below shall be prorated  
to the package sizes shown below:

23	Corn:	80,000 kernel package
	Sorghum:	50 pound package
25	Soybean:	50 pound package
	Canola/Rape:	50 pound package
27	Sunflower:	200,000 kernel package

29 For clarity, an example is provided in Attachment B.1. The numbers used in the example are  
examples and should not be considered binding. In the absence of agreement within sixty (60) days  
31 of commencing negotiations hereunder with respect to any issue in this Exhibit B, any such dispute  
shall be resolved pursuant to Section 8.13.2.

Attachment B.1

Example of Royalty Calculation

Assumptions:

Crop species: Corn  
Total Market Size: 22,000,000 Units  
Licensee Unit Sales: 12,500,000 Units (all units)  
Replant Units: 75,000 (non-royalty bearing)  
Sample Units: 200,000 (royalty bearing)  
Full-Price Units: 3,200,000 (royalty bearing)  
Base Year: 2003 (first year of Commercial Sale)  
Royalty Base Adjustment Factor (for 1997-2001): 3.79% per year  
Single Use Royalty Rate

Calculations:

Licensee Marketshare:  $12,500,000 \text{ Units} / 22,000,000 \text{ Units} = 56.8\%$   
Royalty Rate: .35%  
Qualifying Units: 3,400,000 (sum of Full-Price Units and Sample Units)  
Adjusted Net Royalty Base: See Attachment B.2  
Net Sales:  $\$105.91/\text{Units} * 3,400,000 \text{ Units} = \$360,094,000$

The royalty due and payable for the year 2003 would be:  
 $\$360,094,000 * .35\% = \$1,260,329$

Attachment B.2

Example of  
Adjusted Net Royalty Base  
(for Corn)

Year	Royalty Base Adjustment Factor	Royalty Base	Net to Gross Factor	Adjusted Net Royalty Base
1997	N/A	\$ 105.90	20%	\$ 84.72
1998	3.79%	109.91	20%	87.93
1999	3.79%	114.08	20%	91.26
2000	3.79%	118.40	20%	94.72
2001	3.79%	122.89	20%	98.31
2002	3.79%	127.55	20%	102.04
2003	3.79%	132.38	20%	105.91
2004	3.79%	137.40	20%	109.92
2005	3.79%	142.61	20%	114.09
2006	3.79%	148.01	20%	118.41

Attachment B.3

Commercially Prepared Reports

Merrits and Doans

001020

1 EXHIBIT C

3 Examples of Calculation of License Fee

5 **EXAMPLE 1**

7 Assumption:

9 Value of Licensed Genetic Material = 30

Value of other germ plasm = 70

11 Therefore, percentage of Licensed Genetic Material = 30%

13 Calculation:

15 Factor to apply to License Revenues:  $30\%/2 = 15\%$

001021



*file***Fax Cover Sheet**

DATE: October 16, 1996      TIME: 1:38 PM  
TO: Dr. Ramesh Kumar      TELEPHONE: 215/504-4444  
FAX: 215/504-4545  
FROM: G.D. May      PHONE: 607-254-1268  
BTI      FAX: 607-254-1242  
RE: MTA  
CC:

Number of pages including cover sheet: 7

**Message**

Dear Ramesh,

Please find attached the signed copy of the MTA agreement between BTI and Kimeragen.  
You should receive the original on Thursday by express courier.

Thank you!

All the best!

Greg

001143

## MATERIAL TRANSFER AGREEMENT

This Material Transfer Agreement ("Agreement"), dated October 14, 1996 (the "Effective Date"), is made by and between Kimeragen, Inc., a company incorporated under the laws of the State of Delaware and having an address at 300 Pheasant Run, Newtown, PA 18940 ("Kimeragen") and the Boyce Thompson Institute of Plant Research, Inc., having an address at Tower Road, Cornell University, Ithaca, New York, 14853-1801 (the "Recipient").

WHEREAS, the Recipient desires to obtain certain biological material from Kimeragen for research purposes and Kimeragen desires to supply such material for those purposes.

NOW, THEREFORE in consideration of the mutual covenants hereinafter set out and for good and valuable consideration, the receipt and sufficiency of which is hereby acknowledged, the parties agree as follows:

- I. **The Material and the Recipient:** The Material transferred by Kimeragen comprises chimeric molecules of DNA and RNA as further specified in Part I of Attachment A and such other material as specified in Part II of Attachment A (referred to herein, together with any progeny of, or substances derived or produced from, originating from, made using, or which incorporate any element or portion of that material or its progeny as "Material"). The Recipient intends to use the Material under suitable containment conditions, for research relating to use of the Material in plants (being multicellular rooted organisms containing chlorophyll and cellulose cell walls) as more specifically described in Part III of Attachment A (the "Research Purpose").
- II. **Terms and Conditions:**
  - 2.1 **Ownership:** Kimeragen owns all Material and retains ownership of all Material, notwithstanding that such Material may be in the possession of Recipient or may be subject to modification by Recipient. The Material is or may be the subject of a patent, patent application, trade secret or other proprietary rights of Kimeragen. Kimeragen hereby grants Recipient a non-exclusive sub-license under such rights to use the Material solely in connection with the Research Purpose and subject to the conditions set out in clause 2.2.
  - 2.2 **Use of Material:** The Recipient agrees that Material is to be used only by the Recipient in the laboratory and under the direction of Dr Charles Arntzen and will not be transferred to anyone else within or outside of the Recipient organization without the prior written consent of Kimeragen. The Material is to be used solely for the Research Purpose and not for any activity (whether conducted in the United States or in any other country) which includes, or is for the purpose of, or is intended to result in, (i) the sale, lease, license, or other transfer of Material or material derived or produced therefrom or rights to the use any such material, to any third party; (ii) use of Material by any person, to perform contract research or

to produce or manufacture products for sale; (iii) conduct of research activities for the purpose of, or intended to result in any sale, lease, license or transfer of Material or rights to the use of any such Material, whether or not any such transaction results in fact; (iv) all uses of Material which require any regulatory approval, including without limitation any approval from the United States Food and Drug Administration or any equivalent regulatory body in any foreign country; (v) all uses of Material outside the scope of the Research Purpose in agronomy, in gene therapy, or to produce or otherwise develop transgenic animals; and (vi) all uses of Material in human subjects, in clinical trials or for diagnostic purposes including human subjects whether conducted in the United States or in any foreign country; but nothing in this Agreement shall be taken to prohibit any activity permitted under 35 U.S.C. § 271(e).

- 2.3 **Transfer Fee:** There shall be no transfer fee for the rights granted under this Agreement.
- 2.4 **Confidential Information:** Recipient agrees to maintain the confidentiality of any proprietary information comprised in or otherwise relating to any Material and not to use such information other than for the Research Purpose. The foregoing restrictions under this clause do not apply to any part of the information that is or later becomes available to the public through no breach of this Agreement by Recipient.
- 2.5 **Disclaimer of Warranties and Liability:** Any Material delivered pursuant to this Agreement is understood to be experimental and is provided hereunder as a service to the research community. KIMERAGEN MAKES NO REPRESENTATIONS AND EXTENDS NO WARRANTIES OF ANY KIND, WHETHER EXPRESS OR IMPLIED WITH RESPECT TO THE MATERIAL OR ITS PERFORMANCE. WITHOUT LIMITING THE FOREGOING, THERE ARE NO EXPRESS OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR THAT THE USE OF THE MATERIAL WILL NOT INFRINGE ANY PATENT OR OTHER PROPRIETARY RIGHTS. The Recipient assumes all liability for damages which may arise from its use, storage or disposal of Material. Kimeragen will not be liable to the Recipient or any other person for any loss, claim or demand made by any other party due to, or arising from the use of any Material by the Recipient.
- 2.6 **Intellectual Property** Ownership of inventions (whether patentable or non-patentable), know-how, trade secrets, tangible research property (including biological material), technical data and research results generated or otherwise gained from the research conducted with the Material pursuant to this Agreement (collectively "Research Results") shall be as provided in the Research Collaboration Agreement to be executed between Kimeragen and Recipient. If for any reason, the parties do not enter into such Research Collaboration Agreement, the parties agree that in consideration for the rights granted to Recipient hereunder and certain payments and royalties as set out in Attachment B hereto, all Research Results shall be owned by Kimeragen and the parties shall enter into such agreements (including the terms set out on Attachment B)

and do all other acts or things reasonably necessary to give effect to that intention.

**III. Termination of Agreement:**

- 3.1 This Agreement will terminate on completion of the Research Purpose; or immediately upon written notice from Kimeragen to Recipient of any material breach of this Agreement by Recipient. Notwithstanding the provisions of the previous clause, clauses 2.2, 2.4, 2.5, 3.2 and 4.4 and the obligations of the parties arising therefrom shall survive termination of this Agreement.
- 3.2 Upon the effective date of termination, Recipient will discontinue use of all Material immediately and will, upon direction of Kimeragen, return or destroy any remaining Material unless permitted to continue use of that material under a separate written agreement with Kimeragen.

**IV. Miscellaneous:**

- 4.1 **Use of Name:** Recipient shall not use the name of Kimeragen or TJU or the names of any of their respective staff members, employees or students or any adaptation thereof in any advertising, promotional or sales literature to the extent such use might imply a relationship between the parties, or endorsement by Kimeragen or TJU of any act or thing done by Recipient or of any product or method described in such literature, without the prior written consent of Kimeragen and TJU, which consent shall not be unreasonably withheld.
- 4.2 **Choice of Law:** This Agreement shall be governed by and construed in accordance with the laws of the State of New York, without giving effect to any choice of law or conflict of law provision.
- 4.3 **Severability:** If any provision of this Agreement is held or declared to be illegal, invalid or unenforceable, the remaining provisions of this Agreement shall not be affected and shall continue in full force and effect.
- 4.4 **Assignment of Agreement:** This agreement shall not be assignable by the Recipient without the prior written consent of Kimeragen or its successors or assignees. Any purported assignment by Recipient in contravention of this clause 4.4 shall be void.
- 4.5 **Entire Agreement:** This Agreement sets forth the entire understanding and agreement between Kimeragen and Recipient as to the subject matter set forth herein.

IN WITNESS WHEREOF, the parties have caused this Agreement to be executed by their duly authorized representatives as of this 14 day of October, 1996.

KIMERAGEN, INC.

BOYCE THOMPSON INSTITUTE OF PLANT RESEARCH, INC.

By: [Signature]  
Name: RAMESH KUMAR  
Title: V.P. Technology & Product Development

By: [Signature]  
Name: GREGORY D. MAY  
Title: Assistant Research Scientist

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# Attachment A

Fluorescently labeled Chimera Oligonucleotide AP-2M of the following sequence:

AP-2M (68 mer)

5' A GCG CCG CCT ACG CCC ACT CCG

CTG TTTT cag ccg agc ggg TGT

agg cgg cgc ugc ggc TTTT CAC GC 3'

[where underlined (lower case) letters represent 2'-O Me RNA]

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**Attachment B**

I. Kimeragen shall pay to the Institute a royalty of one percent (1%) of the net sales price of products that are covered by a valid claim of an assigned patent up to a cumulative maximum royalty base of three hundred million dollars (U.S. \$300,000,000) in net sales, provided that in the event that such products are subject to royalties under license and other agreements with third parties, the royalty payable to the Institute shall be reduced so that the total royalty burden on such products shall not exceed one percent (1%), provided further that the royalty payable to the Institute shall in no event be less than one quarter percent ( $\frac{1}{4}\%$ ). Royalties shall be payable on a country by country basis until the expiration of the last valid claim covering a product in any country in which a product is made, used or sold.

II. Kimeragen shall pay to the Institute a percentage of any revenues received from third party licensees equal to ten percent (10%) of revenue covered by a valid claim of an assigned patent, subject to reduction as provided in paragraph 3 hereof, but in no event less than two and one half ( $2\frac{1}{2}\%$ ).

III. Provided that products are developed that are protected by a valid U.S. patent claim under an assigned patent, Kimeragen shall make payments to the Institute at the beginning of each year, which shall be creditable in full against running royalties. Such fees shall be as follows:

(a) Five thousand U.S. dollars (US\$5,000) upon execution of the assignment;

(b) Five thousand U.S. dollars (US\$5,000) on or before each anniversary of the date of the assignment.

IV. Kimeragen shall pay for and control the filing of patent applications and shall reimburse the Institute for any filing fees and reasonable attorneys fees incurred by the Institute in filing, prosecuting and/or maintaining any patent application and/or patent which has been assigned to Kimeragen.

V. The Institute shall provide Kimeragen with such assistance as Kimeragen reasonably requests, including by execution of documents and access to employees and/or contractors of the Institute to enable patent applications for any Research Results to be filed and maintained, and to assist in the conduct and/or defense of any litigation with respect to or in connection with any Research Results.

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KIMERAGEN, INC.

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RESEARCH COLLABORATION AGREEMENT

This Research Collaboration Agreement ("Agreement") is made by and between Kimeragen, Inc., a Delaware corporation, having an address at 300 Pheasant Run, Newtown, PA 18940 (the "Company") and the Boyce Thompson Institute of Plant Research, Inc. of Tower Road, Cornell University, Ithaca, New York 14853-1801 (the "Institute") effective as of October \_\_, 1996 (the "Effective Date").

WHEREAS, the Company and the Institute have agreed that the Institute will conduct research for, and engage in collaborative research with, the Company under the direction of <1> Drs. Hugh Mason and Greg May, as principal investigators (the "Investigators") on the terms set forth in this Agreement.

NOW, THEREFORE, in consideration of the mutual covenants hereinafter set out and for good and valuable consideration, the receipt and sufficiency of which is hereby acknowledged, the parties agree as follows:

1. RESEARCH

(a) The Institute shall perform the research program set forth in Schedule 1 hereto and each such other research program(s) and/or modification(s) thereof, a written description of which is marked with a statement signed by the parties hereto as a research program for the purposes of this Agreement (each a "Research Program"), under the direction of the <2> Investigators, for and in collaboration with the Company in accordance with the terms and conditions of this Agreement. Each Research Program shall include the agreed term of, and budget for, that Research Program. The Institute shall ensure that the highest standards are observed in conducting each Research Program.

(b) The Institute shall keep the Company advised as to the progress in performing each Research Program (including by making prompt written disclosure of all Research Results (as defined in Clause 5(a), below) and the Institute will, as requested by the Company, prepare any additional written reports with respect thereto. The Institute shall prepare a final report for each Research Program within sixty (60) days of completion of the Research Program. Subject to Clause 3, reports prepared for the Company shall be the sole property of the Company.

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2. COMPENSATION FOR RESEARCH COSTS

The Company shall reimburse the Institute for its reasonable costs incurred in conduct of Research Programs in accordance with the budget agreed between the parties for the Research Program in each case. Unless otherwise agreed in the budget, payments from the Company shall not be due more frequently than quarterly. The Institute shall apply all payments received from the Company in accordance with the agreed budget for the applicable Research Program. Any funds paid by the Company and not used by the Institute in the Research Program for which they were paid in any contract year shall be applied against payments due by the Company for that Research Program in the subsequent contract year, or, if that Research Program has been completed or terminated, shall be refunded to the Company or applied against amounts due by the Company to the Institute for any other current Research Program, at the Company's option.

3. PUBLICATION

The <3> Investigators and other employees or contractors of the Institute that are staffed on any Research Program shall be entitled to publish or disclose research results or other material generated in performance of that Research Program, provided that the Institute shall: (1) provide a draft of the disclosure to the Company at least thirty (30) days in advance of submitting such disclosure for proposed publication or presentation; and (2) acknowledge the Company in the publication or disclosure as the collaborator in the Research Program. The Institute shall cause its employees and its contractors to make all reasonable changes requested by the Company. If the Company notifies the Institute that it considers that a proposed publication or presentation includes material which may be patentable, then the Institute shall cause its employees and its contractors to delay the proposed publication or presentation for at least an additional sixty (60) days or such longer period as agreed upon by the parties to permit a patent application with respect to that subject matter to be prepared and filed.

4. CONFIDENTIALITY AND MATERIAL TRANSFER

(a) In performing or otherwise in connection with each Research Program, (i) the Institute may acquire, receive, observe or generate, alone or jointly with others, information and/or material (including certain Biological Material as defined in this Clause 4) that is confidential or proprietary information of the Company ("Company Proprietary Information"); (ii) the Company may acquire, receive or observe information and/or material that is confidential or proprietary to the Institute ("Institute Proprietary Information"); and (iii) confidential information and/or material may be generated by persons carrying out the Research Program ("Research Information"). Subject to Clause 3, the Institute agrees not to disclose any Company Proprietary Information or Research Information to any third party or to use any Company Proprietary Information for any purpose other than performance of Research Programs pursuant to this Agreement, without prior written consent of the Company. The Company agrees not to disclose Institute Proprietary Information or

Research Information to any third party without the Institute's written consent, provided, however, that the Company may disclose Research Information for any business purposes, including without limitation, for the purposes of developing and/or commercializing any technology or other subject matter assigned or licensed to it under this agreement or any other agreement between the parties. The Company Proprietary Information, Institute Proprietary Information and Research Information shall be referred to collectively as "Proprietary Information."

(b) Proprietary Information does not include information which: (i) is or later becomes available to the public through no breach of this Agreement; (ii) is obtained by the receiving party from a third party who had the legal right to possess and disclose the information to that party and is not associated with the Research Program to which the Proprietary Information relates; (iii) the receiving party can show was already in its possession prior to (direct or indirect) receipt by the receiving party from, or disclosure to the receiving party by, the disclosing party; or (iv) is required to be disclosed under any applicable law or court order provided that the receiving party provides the disclosing party with prior written notice of the requirement for disclosure detailing the Proprietary Information to be disclosed, to enable the disclosing party to seek a protective order or otherwise prevent disclosure of such information.

(c) In the course of performing Research Programs hereunder, the Institute may receive from the Company biological material, (including without limitation cell cultures, organisms, proteins, nucleic acids, and/or other chemical or biochemical materials) ("Biological Material"). The Institute shall use the Biological Material only for the purposes of performing the Research Program in connection with which the Biological Material was provided, and shall not provide any Biological Material to any third party unless authorized in writing by the Company to do so, and then only in accordance with the Company's procedures for transfer of such Biological Material. All progeny of, modifications to, material derived or produced from, originating from, made using or which incorporate any element of any Biological Material or progeny thereof, or otherwise made in performance of any Research Program shall be included within the definition of Biological Material for the purposes of this Clause 4. The Biological Material is proprietary to the Company and shall remain the property of the Company notwithstanding that the Biological Material may be or come into the possession of the Institute and/or <4> Investigators, and shall not be removed from the location designated by the Company for performance of the Research Program in connection with which the Biological Material was provided, without the Company's prior written consent.

(d) Following the termination of this Agreement or upon earlier request by the Company, the Institute shall promptly deliver all materials comprising or including any Company Proprietary Information including but not limited to, all written materials, computer media and Biological Material in its possession; and shall delete all Company Proprietary Information from computer disks or electronic storage facilities owned or used by the Institute and/or its employees and/or contractors.

5. OWNERSHIP OF INTELLECTUAL PROPERTY

(a) Subject to paragraph (b), below, all rights in and title to any and all inventions (whether patentable or non-patentable), know-how, trade secrets, tangible research property (including biological material), technical data and research results generated or otherwise gained from any Research Program (collectively "Research Results") and, to the extent applicable, any patent applications or patents covering any Research Results ("Research Patents") conceived or developed or applied for in the course of, or as the result of, conducting any Research Program shall be the property of the Company. The Company shall be responsible, at its cost, for the preparation, filing, prosecution and maintenance of any Research patents. The Institute shall disclose all Research Results to the Company promptly in writing as provided in Clause 1(b).

(b) The rights granted to the Company under Clause 5(a) shall be subject to any prior rights which the U.S. Government or any agency thereof may have in and to Research patents as a result of 35 U.S.C. § 200 *et seq.* and 37 C.F.R. § 401 or otherwise. The Institute shall elect title to any Research Patent to which this Clause 5(b) applies pursuant to 35 U.S.C. § 202.

(c) The Institute hereby assigns to the Company all rights and title it may have to any Research Results and/or Research Patents and undertakes that it shall, and shall cause its employees and contractors (as required) to take all steps and actions and execute and record all documents necessary to record the assignment of the Research Results (and Research Patents, if any) and otherwise give effect to this Clause 5. Without limiting the foregoing, the Institute shall and shall cause its employees and contractors to provide all assistance reasonably required by the Company to give effect to this Clause 5 and enable the Company to prosecute and maintain Research Patents.

(d) The Company hereby grants the Institute a perpetual, royalty-free, worldwide license to practice under Research Patents for its own non-commercial research purposes. In consideration for that license, the Institute hereby grants the Company an exclusive option to any and all inventions (whether patentable or non-patentable), know-how, trade secrets, tangible research property (including biological material), technical data and research results generated or otherwise gained from any such licensed research and owned by the Institute (collectively "Licensed Research Results"), and, to the extent applicable, all patent applications and patents covering any such Licensed Research Results. The option granted herein shall extend for twenty-four (24) months from the Company being notified by the Institute of such Licensed Research Results. The option granted hereunder shall be exercisable at any time, or from time to time, during that option period by notice to the Institute in writing. Following exercise of the option by the Company such Licensed Research Results shall be treated as part of the Research Results and such patents or patent applications covering such Licensed Research Results shall be treated as Research Patents, and accordingly the consideration for the exercise of that option shall be as set out in Clause 6. Following exercise of the option, the parties shall take all steps

**AGREEMENT**

This Agreement is made on \_\_\_\_\_, 1996 by and between Kimeragen, Inc., a company incorporated in Delaware having an address at 300 Pheasant Run, Newtown, PA 18940 ("Kimeragen") and The Jackson Laboratory having an address at 600 Main Street, Bar Harbor, Maine 04609-1500 ("Jackson Laboratory").

WHEREAS, Kimeragen is the exclusive licensee of Thomas Jefferson University ("TJU") under U.S. and foreign patent applications with respect to a chimeric vector for application in gene therapy developed at TJU.

WHEREAS, Jackson Laboratory has agreed to produce transgenic mice for Kimeragen using certain chimeric vectors (as described more fully herein) and related technology.

NOW, THEREFORE, in consideration of the mutual rights and obligations set out in this Agreement and for other good and valuable consideration, the receipt of which is hereby acknowledged, the parties agree as follows:

**1. Definitions**

As used in this Agreement, the following terms shall have the meanings ascribed to them below:

**"Batch"** means mice generated from one microinjection session using Mutation Chimera.

**"Chimera"** means chimeric vectors of RNA and DNA utilized for *ex vivo* or *in vivo* genetic repair, genetic therapy or other cellular or biological applications.

**"Mutation Chimera"** means Chimera having the certain nucleotide sequence described in Part A of Schedule 1 to this Agreement.

**"Patent Rights"** means rights in and to (i) the subject matter of U.S. Patent No. 5,565,350 entitled "Compounds and Methods for Site Directed Mutations in Eukaryotic Cells"; U.S. Patent Application Serial No. 08/644,517 filed on May 1, 1996 entitled "Methods and Compounds for Curing Diseases Caused by Mutations"; and U.S. Patent Application Serial No. 08/664,487 filed on June 17, 1996 entitled "Chimeric Mutational Vectors Having Non-natural Nucleotides" (ii) any future patent applications with respect to Chimera; and (iii) any divisions, continuations or continuations-in-part of (i) or (ii), all foreign

counterparts of (i) or (ii), any patents that may issue from any of the foregoing (including without limitation (i) or (ii)) and all reissues, extensions or re-examinations of any such patents.

**"Technical Information"** means unpatented inventions (whether or not patentable), know-how, trade secrets, tangible research property (including without limitation biological material), technical data, specifications and research results, and includes any unpublished patent applications.

**2. Development and Supply of Chimera**

(a) Kimeragen will develop Mutation Chimera intended for use to produce a [pax 6] mutation in mice.

(b) Kimeragen will supply or arrange the supply of Mutation Chimera to Jackson Laboratory in quantities reasonably required by Jackson Laboratory for the purposes described in clause 3. Kimeragen shall provide Jackson Laboratory with such Technical Information relating to use of Chimera as Kimeragen considers reasonably required for the purposes described in clause 3.

(c) Kimeragen shall retain ownership of all Mutation Chimera and any other biological material provided by Kimeragen to Jackson Laboratory for the purposes of this Agreement. All Mutation Chimera and any other biological material provided by Kimeragen to Jackson Laboratory for the purposes of this Agreement not used at the time Jackson Laboratory receives notification from Kimeragen under clause 3(c) that it does not require any further Batches shall, at Kimeragen's option, be delivered to Kimeragen or destroyed. All other material existing at that time which was derived from, made using or which incorporates Mutation Chimera or other biological material provided to Jackson Laboratory by Kimeragen shall be destroyed. Jackson Laboratory shall not provide any Mutation Chimera or other biological material received from Kimeragen or any other material derived from, made using or which incorporates such Mutation Chimera or biological material to any third party without Kimeragen's written consent.

**3. Development and Supply of Transgenic Mice**

(a) Jackson Laboratory shall use best efforts to produce transgenic mice which have a [pax 6] mutation and otherwise meet the specifications provided by Kimeragen, including, without limitation, the specifications set out in Part B of Schedule 1 to this Agreement using the Mutation Chimera.

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(b) Jackson Laboratory shall use best efforts to produce the first Batch within **[five (5) weeks]** of the date of this Agreement.

(c) Jackson Laboratory shall produce not less than four (4) and up to twelve (12) Batches for Kimeragen. Kimeragen may instruct Jackson Laboratory to discontinue production after the fourth or any subsequent Batch, provided, however, that Jackson Laboratory may complete production of any Batch for which production has commenced at the time Jackson Laboratory receives such notification from Kimeragen.

(d) Kimeragen shall own the mice produced for it under this Agreement, and shall have the right to breed such mice. Jackson Laboratory shall deliver mice produced for Kimeragen under this Agreement at Kimeragen's request to the location(s) designated by Kimeragen and in accordance with other reasonable instructions given by Kimeragen for delivery.

**4. License**

Kimeragen hereby grants Jackson Laboratory a non-exclusive, royalty free sub-license under the Patent Rights and related Technical Information to the extent reasonably necessary to enable Jackson Laboratory to produce the transgenic mice for Kimeragen under this Agreement. Jackson Laboratory shall not have any right to use the Chimera or practice any Patent Rights or Technical Information relating to Chimera except as set out in this clause 4 or pursuant to a separate written license agreement.

**5. Payment**

Kimeragen shall pay Jackson Laboratory \$[ ] per Batch. Payment shall be due **[ terms]**.

**6. Ongoing Negotiation**

Kimeragen agrees to negotiate in good faith with Jackson Laboratory to determine whether the parties are able to reach mutually acceptable terms with respect to a further and separate license agreement for use by Jackson Laboratory of Patent Rights and related Kimeragen Technical Information to produce transgenic mice and rats.

**7. Technical Information**

(a) Jackson Laboratory and Kimeragen recognize that they may receive and/or generate Technical Information in connection with the activities conducted under this Agreement. Each party agrees (a) not to disclose any Technical Information disclosed or

provided to it by the other party to any third party, without the express written consent of the disclosing party; and (b) not to use any Technical Information provided to it by the other party except for the purposes contemplated by this Agreement. These obligations of confidence do not extend to any part of the Technical Information which (i) is agreed in writing and without restriction by the parties to be excluded; or (ii) the receiving party can prove by written records was known to or developed by it prior to the date of first disclosure of any Technical Information to it by the disclosing party, and was not generated in the course of performance under this Agreement; or (iii) which is public knowledge or becomes public knowledge in the future other than through acts or omissions of the receiving party in breach of its obligations of confidence; or (iv) which the receiving party obtains lawfully from sources independent of the disclosing party who have a lawful right to possess and disclose such information; or (v) is required to be disclosed under any applicable law or regulation provided that the receiving party first notifies the disclosing party in writing of the obligation to make disclosure and the Technical Information to be disclosed and affords the disclosing party an opportunity to seek a protective order or otherwise prevent disclosure of such Technical Information.

(b) Following the termination of this Agreement or upon earlier request, the receiving party shall promptly deliver to the disclosing party all materials comprising Technical Information received from that party and shall delete all such Technical Information and any part thereof from any computer disk or electronic storage facility on which it is stored, and which the receiving party owns or uses. Notwithstanding the foregoing, the receiving party shall be permitted to retain one (1) copy of the Technical Information disclosed to it by the other party, in confidential files, for the sole and exclusive purpose of enabling the receiving party to determine any continuing obligations of confidentiality.

(c) Jackson Laboratory and Kimeragen agree that (i) any Technical Information generated in the course of performance of this Agreement with respect to Chimera or use of Chimera (including use of Chimera in production of transgenic animals) shall be owned by Kimeragen; and (ii) any Technical Information generated in the course of performance of this Agreement with respect to production of transgenic animals and which does not relate to Chimera or use of Chimera shall be owned by Jackson Laboratory. Each party hereby assigns to the other any and all right, title and interest it may have in Technical Information agreed to be owned by the other and agrees to execute all documents reasonably necessary to give effect to this clause 7(c). All Technical Information owned by each party under this clause 7(c) shall be deemed Technical Information disclosed by that party to the other party for the purposes of paragraph (a) of this clause 7. Without limiting the foregoing, each party shall be entitled to apply, at its cost, for patents for any patentable inventions among the Technical Information owned by it hereunder.

**8. Publication**

Subject to clause 7, the parties shall each be entitled to publish or present research results or other material which results from the work carried out under this Agreement, provided that for all publications (i) appropriate persons from Jackson Laboratory and Kimeragen are given the opportunity to review and are named as co-authors of any paper published by either party; and (ii) each party receives acknowledgment for its role in the work conducted under this Agreement. Jackson Laboratory shall provide a summary of any material for presentation to Kimeragen at least thirty (30) days in advance of submitting such material for the purposes of or making such presentation. If Kimeragen notifies Jackson Laboratory that it considers that a proposed publication or presentation includes material which may be patentable, then Jackson Laboratory shall delay the proposed publication or presentation for at least an additional sixty (60) days or such longer period as agreed upon by the parties to permit a patent application to be prepared and filed with respect to that subject matter.

**9. Disclaimer of Warranties**

Any Chimera, including without limitation Mutation Chimera, and any other material delivered to Jackson Laboratory by Kimeragen and any mice delivered to Kimeragen by Jackson Laboratory hereunder are understood to be experimental in nature. THE PARTIES MAKE NO REPRESENTATIONS AND EXTEND NO WARRANTIES OF ANY KIND, WHETHER EXPRESS OR IMPLIED. THERE ARE NO EXPRESS OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR THAT THE USE OF CHIMERA OR RELATED TECHNICAL INFORMATION WILL NOT INFRINGE ANY PROPRIETARY RIGHTS.

**10. Employees of Jackson Laboratory**

Jackson Laboratory represents and warrants that each employee of Jackson Laboratory and each independent contractor of Jackson Laboratory, if any, has executed an agreement or agreements which enable it to fulfill its obligations under this Agreement with respect to any and all parts of the service under this Agreement which are performed by such employees or independent contractors, whether alone or jointly with others.

**11. Term and Termination**

(a) This Agreement shall have a term of [six (6)] months from the Effective Date unless terminated earlier pursuant to paragraph (b) of this clause 11.



(b) Either party may terminate this Agreement effective immediately on written notice to the other if the other commits any material breach of this Agreement and does not remedy such breach within fifteen (15) days of receipt of written notice from the aggrieved party.

## 12. Miscellaneous

(a) Choice of Law. This Agreement shall be governed by and construed in accordance with the domestic laws of the State of New York, without giving effect to any choice of law or conflict of law provision or rule and each party hereby submits to, and acknowledges the jurisdiction of, the courts of the Southern District of State of New York in connection with this Agreement.

(b) Severability. The provisions of this Agreement shall be severable, and if any provision of this Agreement is held or declared to be illegal, invalid or unenforceable, the remaining provisions of this Agreement shall not be affected and shall continue in full force and effect.

(c) Assignment of Agreement. This Agreement shall be assignable to, and upon assignment, shall inure to the benefit of Kimeragen's successors or assignees. This Agreement shall not be assignable by the Jackson Laboratory without the prior written consent of Kimeragen or its successors or assignees. Any purported assignment by Jackson Laboratory in contravention of this clause 6(d) shall be void.

(d) Notices. Any notices, consents or approvals required or permitted to be given hereunder shall be deemed to be given and sufficient when delivered in writing, first class United States certified or registered letter, return receipt requested, or by overnight delivery or courier service or by facsimile with written confirmation as provided above:

to Kimeragen: 300 Pheasant Run  
New Town, PA 18940  
Attention: Vice President Technology and  
Product Development

to Jackson Laboratory: 600 Main Street  
Bar Harbor, Maine 04609-1500  
Attention: House Counsel

(e) Entire Agreement. This Agreement sets forth the entire understanding and agreement between the parties with respect to the subject matter set forth herein.

(f) Nature of Relationship. The parties are independent contractors and not agents or employees of one another. Neither party shall enter into any agreement or incur any obligations on the other's behalf, or commit the other in any manner without the other's prior consent.

(g) Terms Confidential. The terms of this Agreement are confidential and shall not be disclosed by either party to any third party other than their legal advisors and financial advisors without the consent of the other party unless disclosure is required by applicable law or regulation.

IN WITNESS WHEREOF, the parties have caused this Agreement to be executed by their duly authorized representatives as of this \_\_\_\_ day of \_\_\_\_\_, 1996.

**KIMERAGEN, INC.**

By: \_\_\_\_\_

Name: \_\_\_\_\_

Title: \_\_\_\_\_

**THE JACKSON LABORATORY**

By: \_\_\_\_\_

Name: \_\_\_\_\_

Title: \_\_\_\_\_

SCHEDULE A

Part A:      Mutation Chimera

Part B:      Specifications

and execute and record all documents necessary to assign the relevant Research Results and otherwise give effect to this Clause 5(d).

6. ROYALTY OBLIGATIONS

(a) As consideration for the rights granted to the Company under Clause 5, the Company hereby agrees to pay the Institute royalties as follows:

(i) The Company shall pay to the Institute a royalty of one and one-half percent ~~<5>(1½%)~~ of the Net Sales (as defined below) of products that are covered by a valid claim of a Research Patent ~~<6>("Products")~~ up to a cumulative maximum royalty base of three hundred million dollars (U.S. \$300,000,000) in Net Sales, provided that in the event that any ~~<7> Products~~ are subject to royalties under license and other agreements with third parties, the royalty payable to the Institute with respect to such ~~<8> Products~~ shall be reduced so that the total royalty burden on such ~~<9> Products~~ shall not exceed one and one-half percent ~~<10>(1½%)~~, provided further that the royalty payable to the Institute shall in no event be less than three eighths of one ~~<11> percent~~ ~~<12>(¾%)~~.

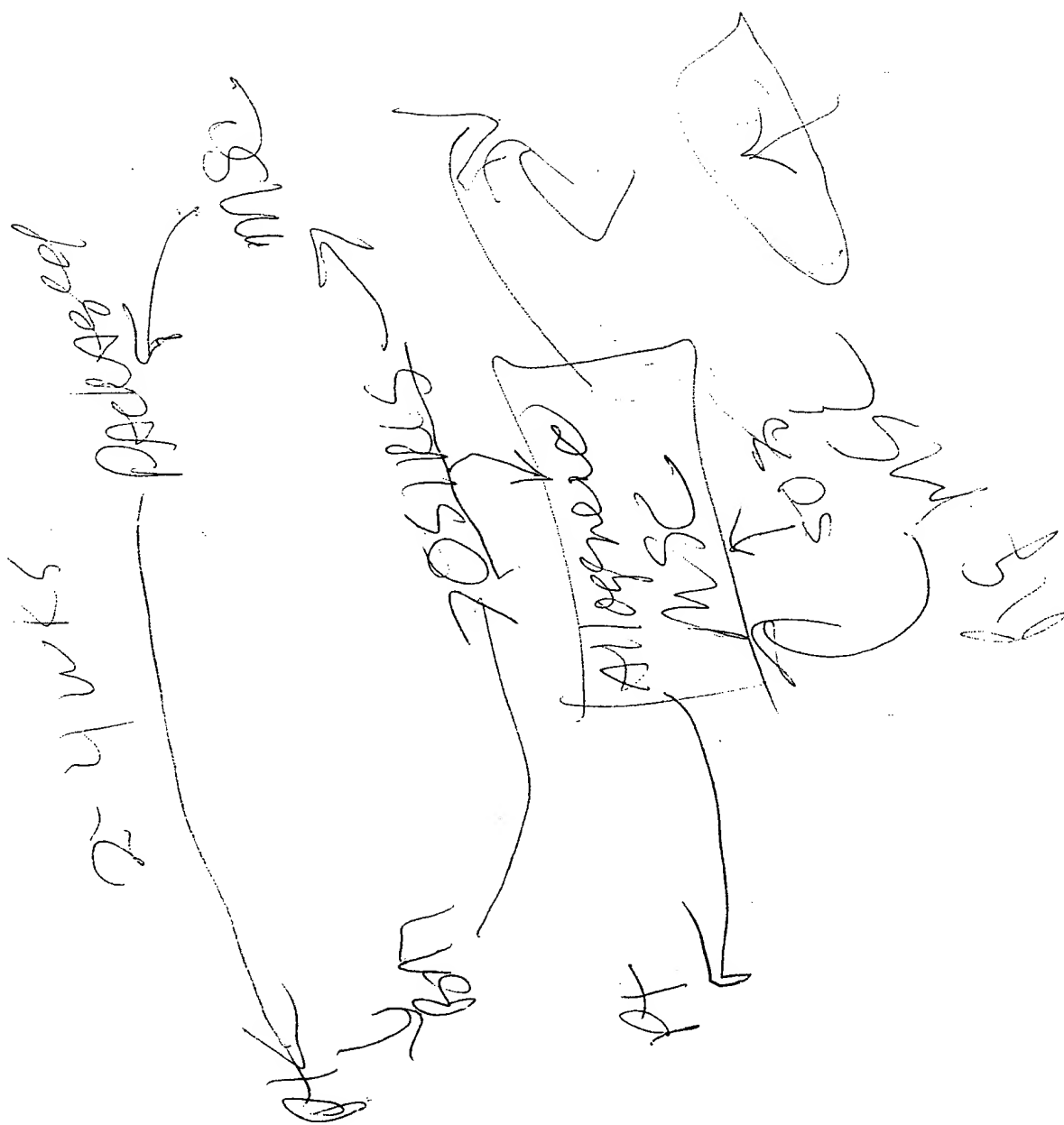
(ii) Royalties shall be payable on a country by country basis on sales of ~~<13> Products~~ (other than sales by the Company to its Subsidiaries or licensees) where the ~~<14> Products~~ were made, used or sold in any country in which there is a valid claim of a Research Patent which covers the ~~<15> Products~~ in question, until the expiration of the last valid claim of a Research Patent in any country in which any ~~<16> Product~~ is made, used or sold.

(iii) The Company shall pay to the Institute a percentage of any revenues received from third party licensees equal to ten percent (10%) of revenue payable by such licensees by reference to a valid claim of a Research Patent, subject to reduction as provided in Clause 6(a)(i) hereof, but in no event less than two and one half (2½%).

(b) Provided that ~~<17> Products~~ are developed that are protected by a valid U.S. patent claim under a Research Patent, the Company shall make payments to the Institute at the beginning of each year following the issuance of the first U.S. Research Patent, which shall be creditable in full against running royalties. Such fees shall be as follows:

(i) Five thousand U.S. dollars (US\$5,000) upon issuance of the first U.S. Research Patent; and

(ii) <18> The greater of (x) five thousand U.S. dollars (US\$5,000); and (y) twenty-five percent (25%) of the total payments made by Kimeragen to the Institute under this clause 6 in the preceding year; on or before each January 31



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of each year thereafter prior to the expiration of the last valid claim of a Research Patent in any country in which any <19> Product is made, used or sold.

(c) Royalties shall be payable by the Company annually in U.S. dollars (net of withholdings and other taxes) within sixty (60) days of the Company's fiscal year end (December 31) after the first commercial sale of any <20> Product. Each royalty payment shall be accompanied by a royalty statement setting out how the royalties were calculated in accordance with this Clause 6. For the purposes of calculation of royalties, payments received from third parties shall be converted into U.S. Dollars according to the exchange rate quoted by the Wall Street Journal effective on the last day of the year to which the payments relate.

(d) For the purposes of this Agreement, "Subsidiary" shall mean, with respect to any person, any other person that, directly or indirectly, controls or is controlled by or is under common control with that first mentioned person. For the purposes of this Clause 6, (i) "Net Sales" shall mean the invoice price ex-works of Products sold by the Company or its Subsidiaries to a customer other than the Company and its Subsidiaries, less any discount, freight, taxes, duties, tariffs, fees and other charges, and after deduction of amounts credited to customers or written off as bad debts; (ii) where any Research Patent is abandoned, expired or held invalid, it shall be treated as if no patent had been issued with respect to that Research Patent.

(e) The Company shall keep proper records of (i) all sales of <21> Products made by the Company; (ii) all sales of <22> Products, all invoices for such sales, and other information necessary for the calculation of Net Sales of <23> Products; (iii) all licenses granted under or with respect to any Research Patents; and (iv) all payments received from third parties under or in respect of any such license. Subject to reasonable prior written notice, the Company shall allow the Institute access to such records during customary business hours for the purposes of verification of royalties paid and payable under this Agreement, provided that the Institute may not have access to such records more than once in any calendar year.

## 7. TERMINATION

(a) The term of this Agreement shall run from the Effective Date until December 31, 1997 or such later date as agreed upon by the parties in writing, provided that either the Institute or the Company may terminate this Agreement by sixty (60) days written notice to the other for any reason or for no reason.

(b) The Institute may terminate this Agreement by fourteen (14) days written notice if the Company commits any material breach of this agreement. The Company may terminate this Agreement by fourteen (14) days written notice if the Institute commits any

material breach of this agreement or if the <24> Investigators resigns from the Institute or becomes unwilling or unable to conduct any Research Program.

(c) Termination or expiration of this Agreement shall not affect: (i) the Company's obligation to pay for research performed under this Agreement in accordance with Clause 2; or (ii) the parties' obligations under Clauses 3 and 4, 5 and 6, above.

8. MISCELLANEOUS

(a) This Agreement shall be deemed to be a contract made under the law of the State of New York applicable to contracts made and performed entirely within the State, and for all purposes this Agreement shall be construed and interpreted in accordance with and be governed by the law of the State of New York.

(b) This Agreement may not be and shall not be deemed or construed to have been modified, amended, rescinded, canceled or waived in whole or in part, except by written instrument signed by the parties. This Agreement, including the Schedules attached hereto, constitutes and expresses the entire agreement and understanding between the parties with respect to its subject matter. Without limiting the foregoing, this Agreement supersedes the material transfer agreement between the parties dated October \_\_, 1996 and all activities conducted under that material transfer agreement shall be considered activities under the initial Research Program for the purposes of this Agreement.

(c) The Institute is an independent contractor and not an agent or partner of the Company. The Institute shall not enter into any agreement or incur any obligations on the Company's behalf, or commit the Company in any manner without the Company's prior consent.

(d) The Institute shall not assign or transfer its obligations under this Agreement without the prior written consent of the Company.

(e) Should any provision of this Agreement be illegal, invalid or unenforceable, all other terms and conditions of this Agreement shall remain in full force and effect.

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IN WITNESS WHEREOF, the parties have executed this Agreement as of the date first set forth above.

BOYCE THOMPSON INSTITUTE OF  
PLANT RESEARCH, INC.:

KIMERAGEN, INC.:

By: \_\_\_\_\_  
Name: \_\_\_\_\_  
Title: \_\_\_\_\_  
Date: \_\_\_\_\_

By: \_\_\_\_\_  
Name: \_\_\_\_\_  
Title: \_\_\_\_\_  
Date: \_\_\_\_\_

I agree to and accept the terms and conditions of this Agreement and agree to act as  
<25> Investigators for the Research Programs established hereunder.

<26> INVESTIGATORS:

INVESTIGATORS:

By: \_\_\_\_\_  
Name: Dr <27> Hugh Mason  
Address: \_\_\_\_\_  
\_\_\_\_\_  
Date: \_\_\_\_\_

By: \_\_\_\_\_  
Name: Dr. Greg May  
Address: \_\_\_\_\_  
\_\_\_\_\_  
Date: \_\_\_\_\_



## SCHEDULE 1

### Research Program

1. See Attachment A.
2. The <28> Investigators shall be available from time to time, subject to reasonable prior notice being received from the Company to discuss and/or make presentations with respect to the performance of the Research Program set out in paragraph 1, above (including without limitation research protocols and methodology used in laboratory work conducted) and the research results generated in performance of the Research Program with representatives of the Company and such other persons as the Company may nominate from time to time, including without limitation potential investors and/or underwriters, the Company's auditors, the Company's patent counsel, media representatives and potential commercial partners and/or customers of the Company.
3. <29> Investigators shall perform such other services relating to the subject matter of the Research Programs and assist the Company with such other matters relating to the subject matter of the Research Programs as are reasonably designated by the Company from time to time during the term of this Agreement.

### Budget

See Attachment A.

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----- COMPARISON OF HEADERS -----

-HEADER 1-  
K&E Draft <30> 11/8/96  
Confidential

----- COMPARISON OF FOOTERS -----

-FOOTER 1-

-FOOTER 2-  
I:\KIMER\AGRMNTS\UNIV\<31> BOYCE.002  
<32> 11/10/96 11:56

001324

## ----- DELETIONS -----

<1> Dr. Charles Amtzen, President and Chief Executive Officer of the Institute, as principal investigator (the "Investigator")

<2> Investigator

<3> Investigator

<4> Investigator

<5> (1%)

<6> ("Licensed

<7> Licensed

<8> Licensed

<9> Licensed products

<10> (1%)

<11> quarter

<12> (¼%)

<13> Licensed

<14> Licensed

<15> Licensed

<16> Licensed

<17> Licensed

<18> Five

<19> Licensed

<20> Licensed

001325

<21> Licensed

<22> Licensed

<23> Licensed

<24> Investigator

<25> Investigator

<26> INVESTIGATOR

<27> Charles Amtzen

<28> Investigator

<29> Investigator

<30> 10/28/96

<31> BOYCE.001

<32> 10/30/96 17:24

001326

**KIRKLAND & ELLIS**  
PARTNERSHIPS INCLUDING PROFESSIONAL CORPORATIONS

Stephen P. H. Johnson  
To Call Writer Direct:  
212 446-4920

Citicorp Center  
153 East 53rd Street  
New York, New York 10022-4875  
212 446-4800

Facsimile:  
212 446-4900

December 5, 1996

**BY FEDERAL EXPRESS**

Dr. Charles Arntzen  
Boyce Thompson Institute of Plant Research, Inc.  
Tower Road  
Cornell University  
Ithaca, New York 14853-1801

Re: Kimeragen

Dear Charlie:

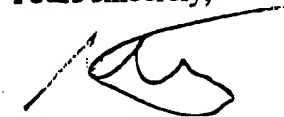
It was a pleasure to meet you at the retreat in Maine.

I enclose four execution copies of the Research Collaboration Agreement. Please will you fill in the date of the MTA in paragraph 8(b). In addition, you and Kimeragen need to fill out the terms of Attachment A.

After signing the agreement, please have the agreements sent to Ramesh Kumar so that they may be executed by Kimeragen.

Please do not hesitate to call if you have any questions.

Yours sincerely,



Stephen P. H. Johnson

SPHJ:dp  
enclosure

cc: Ramesh Kumar, Ph.D.

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**KIMERAGEN, INC.****RESEARCH COLLABORATION AGREEMENT**

This Research Collaboration Agreement ("Agreement") is made by and between Kimeragen, Inc., a Delaware corporation, having an address at 300 Pheasant Run, Newtown, PA 18940 (the "Company") and the Boyce Thompson Institute of Plant Research, Inc. of Tower Road, Cornell University, Ithaca, New York 14853-1801 (the "Institute") effective as of October \_\_, 1996 (the "Effective Date").

WHEREAS, the Company and the Institute have agreed that the Institute will conduct research for, and engage in collaborative research with, the Company under the direction of Drs. Hugh Mason and Greg May, as joint principal investigators (the "Investigators") on the terms set forth in this Agreement.

NOW, THEREFORE, in consideration of the mutual covenants hereinafter set out and for good and valuable consideration, the receipt and sufficiency of which is hereby acknowledged, the parties agree as follows:

**1. RESEARCH**

(a) The Institute shall perform the research program set forth in Schedule 1 hereto and each such other research program(s) and/or modification(s) thereof, a written description of which is marked with a statement signed by the parties hereto as a research program for the purposes of this Agreement (each a "Research Program"), under the direction of the Investigators, for and in collaboration with the Company in accordance with the terms and conditions of this Agreement. Each Research Program shall include the agreed term of, and budget for, that Research Program. The Institute shall ensure that the highest standards are observed in conducting each Research Program.

(b) The Institute shall keep the Company advised as to the progress in performing each Research Program (including by making prompt written disclosure of all Research Results (as defined in Clause 5(a), below) and the Institute will, as requested by the Company, prepare any additional written reports with respect thereto. The Institute shall prepare a final report for each Research Program within sixty (60) days of completion of the Research Program. Subject to Clause 3, reports prepared for the Company shall be the sole property of the Company.

**2. COMPENSATION FOR RESEARCH COSTS**

The Company shall reimburse the Institute for its reasonable costs incurred in conduct of Research Programs in accordance with the budget agreed between the parties for the Research Program in each case. Unless otherwise agreed in the budget, payments from the Company shall not be due more frequently than quarterly. The Institute shall apply all payments received from the Company in accordance with the agreed budget for the applicable Research Program. Any funds paid by the Company and not used by the

Institute in the Research Program for which they were paid in any contract year shall be applied against payments due by the Company for that Research Program in the subsequent contract year, or, if that Research Program has been completed or terminated, shall be refunded to the Company or applied against amounts due by the Company to the Institute for any other current Research Program, at the Company's option.

### 3. PUBLICATION

The Investigators and other employees or contractors of the Institute that are staffed on any Research Program shall be entitled to publish or disclose research results or other material generated in performance of that Research Program, provided that the Institute shall: (1) provide a draft of the disclosure to the Company at least thirty (30) days in advance of submitting such disclosure for proposed publication or presentation; and (2) acknowledge the Company in the publication or disclosure as the collaborator in the Research Program. The Institute shall cause its employees and its contractors to make all reasonable changes requested by the Company. If the Company notifies the Institute that it considers that a proposed publication or presentation includes material which may be patentable, then the Institute shall cause its employees and its contractors to delay the proposed publication or presentation for at least an additional sixty (60) days or such longer period as agreed upon by the parties to permit a patent application with respect to that subject matter to be prepared and filed.

### 4. CONFIDENTIALITY AND MATERIAL TRANSFER

(a) In performing or otherwise in connection with each Research Program, (i) the Institute may acquire, receive, observe or generate, alone or jointly with others, information and/or material (including certain Biological Material as defined in this Clause 4) that is confidential or proprietary information of the Company ("Company Proprietary Information"); (ii) the Company may acquire, receive or observe information and/or material that is confidential or proprietary to the Institute ("Institute Proprietary Information"); and (iii) confidential information and/or material may be generated by persons carrying out the Research Program ("Research Information"). Subject to Clause 3, the Institute agrees not to disclose any Company Proprietary Information or Research Information to any third party or to use any Company Proprietary Information for any purpose other than performance of Research Programs pursuant to this Agreement, without prior written consent of the Company. The Company agrees not to disclose Institute Proprietary Information or Research Information to any third party without the Institute's written consent, provided, however, that the Company may disclose Research Information for any business purposes, including without limitation, for the purposes of developing and/or commercializing any technology or other subject matter assigned or licensed to it under this agreement or any other agreement between the parties. The Company Proprietary Information, Institute Proprietary Information and Research Information shall be referred to collectively as "Proprietary Information."

(b) Proprietary Information does not include information which: (i) is or later becomes available to the public through no breach of this Agreement; (ii) is obtained by the receiving party from a third party who had the legal right to possess and disclose the

information to that party and is not associated with the Research Program to which the Proprietary Information relates; (iii) the receiving party can show was already in its possession prior to (direct or indirect) receipt by the receiving party from, or disclosure to the receiving party by, the disclosing party; or (iv) is required to be disclosed under any applicable law or court order provided that the receiving party provides the disclosing party with prior written notice of the requirement for disclosure detailing the Proprietary Information to be disclosed, to enable the disclosing party to seek a protective order or otherwise prevent disclosure of such information.

(c) In the course of performing Research Programs hereunder, the Institute may receive from the Company biological material, (including without limitation cell cultures, organisms, proteins, nucleic acids, and/or other chemical or biochemical materials) ("Biological Material"). The Institute shall use the Biological Material only for the purposes of performing the Research Program in connection with which the Biological Material was provided, and shall not provide any Biological Material to any third party unless authorized in writing by the Company to do so, and then only in accordance with the Company's procedures for transfer of such Biological Material. All progeny of, modifications to, material derived or produced from, originating from, made using or which incorporate any element of any Biological Material or progeny thereof, or otherwise made in performance of any Research Program shall be included within the definition of Biological Material for the purposes of this Clause 4. The Biological Material is proprietary to the Company and shall remain the property of the Company notwithstanding that the Biological Material may be or come into the possession of the Institute and/or Investigators, and shall not be removed from the location designated by the Company for performance of the Research Program in connection with which the Biological Material was provided, without the Company's prior written consent.

(d) Following the termination of this Agreement or upon earlier request by the Company, the Institute shall promptly deliver all materials comprising or including any Company Proprietary Information including but not limited to, all written materials, computer media and Biological Material in its possession; and shall delete all Company Proprietary Information from computer disks or electronic storage facilities owned or used by the Institute and/or its employees and/or contractors.

## 5. OWNERSHIP OF INTELLECTUAL PROPERTY

(a) Subject to paragraph (b), below, all rights in and title to any and all inventions (whether patentable or non-patentable), know-how, trade secrets, tangible research property (including biological material), technical data and research results generated or otherwise gained from any Research Program (collectively "Research Results") and, to the extent applicable, any patent applications or patents covering any Research Results ("Research Patents") conceived or developed or applied for in the course of, or as the result of, conducting any Research Program shall be the property of the Company. The Company shall be responsible, at its cost, for the preparation, filing, prosecution and maintenance of any Research Patents. The Institute shall disclose all Research Results to the Company promptly in writing as provided in Clause 1(b).



(b) The rights granted to the Company under Clause 5(a) shall be subject to any prior rights which the U.S. Government or any agency thereof may have in and to Research patents as a result of 35 U.S.C. § 200 *et seq.* and 37 C.F.R. § 401 or otherwise. The Institute shall elect title to any Research Patent to which this Clause 5(b) applies pursuant to 35 U.S.C. § 202.

(c) The Institute hereby assigns to the Company all rights and title it may have to any Research Results and/or Research Patents and undertakes that it shall, and shall cause its employees and contractors (as required) to take all steps and actions and execute and record all documents necessary to record the assignment of the Research Results (and Research Patents, if any) and otherwise give effect to this Clause 5. Without limiting the foregoing, the Institute shall and shall cause its employees and contractors to provide all assistance reasonably required by the Company to give effect to this Clause 5 and enable the Company to prosecute and maintain Research Patents.

(d) The Company hereby grants the Institute a perpetual, royalty-free, worldwide license to practice under Research Patents for its own non-commercial research purposes. In consideration for that license, the Institute hereby grants the Company an exclusive option to any and all inventions (whether patentable or non-patentable), know-how, trade secrets, tangible research property (including biological material), technical data and research results generated or otherwise gained from any such licensed research and owned by the Institute (collectively "Licensed Research Results"), and, to the extent applicable, all patent applications and patents covering any such Licensed Research Results. The option granted herein shall extend for twenty-four (24) months from the Company being notified by the Institute of such Licensed Research Results. The option granted hereunder shall be exercisable at any time, or from time to time, during that option period by notice to the Institute in writing. Following exercise of the option by the Company such Licensed Research Results shall be treated as part of the Research Results and such patents or patent applications covering such Licensed Research Results shall be treated as Research Patents, and accordingly the consideration for the exercise of that option shall be as set out in Clause 6. Following exercise of the option, the parties shall take all steps and execute and record all documents necessary to assign the relevant Research Results and otherwise give effect to this Clause 5(d).

## 6. ROYALTY OBLIGATIONS

(a) As consideration for the rights granted to the Company under Clause 5, the Company hereby agrees to pay the Institute royalties as follows:

(i) The Company shall pay to the Institute a royalty of one and one-half percent (1½%) of the Net Sales (as defined below) of products that are covered by a valid claim of a Research Patent ("Products") up to a cumulative maximum royalty base of three hundred million dollars (U.S. \$300,000,000) in Net Sales, provided that in the event that any Products are subject to royalties under license and other agreements with third parties, the royalty payable to the Institute with respect to such Products shall be reduced so that the total royalty burden on such Products shall not exceed one and one-half percent (1½%), provided further that the royalty

payable to the Institute shall in no event be less than one quarter of one percent (¼%).

(ii) Royalties shall be payable on a country by country basis on sales of Products (other than sales by the Company to its Subsidiaries or licensees) where the Products were made, used or sold in any country in which there is a valid claim of a Research Patent which covers the Products in question, until the expiration of the last valid claim of a Research Patent in any country in which any Product is made, used or sold.

(iii) The Company shall pay to the Institute a percentage of any revenues received from third party licensees equal to ten percent (10%) of revenue payable by such licensees by reference to a valid claim of a Research Patent, subject to reduction as provided in Clause 6(a)(i) hereof, but in no event less than two and one half (2½%).

(b) Provided that Products are developed that are protected by a valid U.S. patent claim under a Research Patent, the Company shall make payments to the Institute at the beginning of each year following the issuance of the first U.S. Research Patent, which shall be creditable in full against running royalties. Such fees shall be as follows:

(i) Five thousand U.S. dollars (US\$5,000) upon issuance of the first U.S. Research Patent; and

(ii) The greater of (x) five thousand U.S. dollars (US\$5,000); and (y) twenty-five percent (25%) of the total payments made by Kimeragen to the Institute under this clause 6 in the preceding year; on or before each January 31 of each year thereafter prior to the expiration of the last valid claim of a Research Patent in any country in which any Product is made, used or sold.

(c) Royalties shall be payable by the Company annually in U.S. dollars (net of withholdings and other taxes) within sixty (60) days of the Company's fiscal year end (December 31) after the first commercial sale of any Product. Each royalty payment shall be accompanied by a royalty statement setting out how the royalties were calculated in accordance with this Clause 6. For the purposes of calculation of royalties, payments received from third parties shall be converted into U.S. Dollars according to the exchange rate quoted by the Wall Street Journal effective on the last day of the year to which the payments relate.

(d) For the purposes of this Agreement, "Subsidiary" shall mean, with respect to any person, any other person that, directly or indirectly, controls or is controlled by or is under common control with that first mentioned person. For the purposes of this Clause 6, (i) "Net Sales" shall mean the invoice price ex-works of Products sold by the Company or its Subsidiaries to a customer other than the Company and its Subsidiaries, less any discount, freight, taxes, duties, tariffs, fees and other charges, and after deduction of amounts credited to customers or written off as bad debts; (ii) where any Research Patent

is abandoned, expired or held invalid, it shall be treated as if no patent had been issued with respect to that Research Patent.

(e) The Company shall keep proper records of (i) all sales of Products made by the Company; (ii) all sales of Products, all invoices for such sales, and other information necessary for the calculation of Net Sales of Products; (iii) all licenses granted under or with respect to any Research Patents; and (iv) all payments received from third parties under or in respect of any such license. Subject to reasonable prior written notice, the Company shall allow the Institute access to such records during customary business hours for the purposes of verification of royalties paid and payable under this Agreement, provided that the Institute may not have access to such records more than once in any calendar year.

## 7. TERMINATION

(a) The term of this Agreement shall run from the Effective Date until December 31, 1997 or such later date as agreed upon by the parties in writing, provided that either the Institute or the Company may terminate this Agreement by sixty (60) days written notice to the other for any reason or for no reason.

(b) The Institute may terminate this Agreement by fourteen (14) days written notice if the Company commits any material breach of this agreement. The Company may terminate this Agreement by fourteen (14) days written notice if the Institute commits any material breach of this agreement or if the Investigators resigns from the Institute or becomes unwilling or unable to conduct any Research Program.

(c) Termination or expiration of this Agreement shall not affect: (i) the Company's obligation to pay for research performed under this Agreement in accordance with Clause 2; or (ii) the parties' obligations under Clauses 3 and 4, 5 and 6, above.

## 8. MISCELLANEOUS

(a) This Agreement shall be deemed to be a contract made under the law of the State of New York applicable to contracts made and performed entirely within the State, and for all purposes this Agreement shall be construed and interpreted in accordance with and be governed by the law of the State of New York.

(b) This Agreement may not be and shall not be deemed or construed to have been modified, amended, rescinded, canceled or waived in whole or in part, except by written instrument signed by the parties. This Agreement, including the Schedules attached hereto, constitutes and expresses the entire agreement and understanding between the parties with respect to its subject matter. Without limiting the foregoing, this Agreement supersedes the material transfer agreement between the parties dated October \_\_, 1996 and all activities conducted under that material transfer agreement shall be considered activities under the initial Research Program for the purposes of this Agreement.

(c) The Institute is an independent contractor and not an agent or partner of the Company. The Institute shall not enter into any agreement or incur any obligations on the Company's behalf, or commit the Company in any manner without the Company's prior consent.

(d) The Institute shall not assign or transfer its obligations under this Agreement without the prior written consent of the Company.

(e) Should any provision of this Agreement be illegal, invalid or unenforceable, all other terms and conditions of this Agreement shall remain in full force and effect.

IN WITNESS WHEREOF, the parties have executed this Agreement as of the date first set forth above.

BOYCE THOMPSON INSTITUTE OF  
PLANT RESEARCH, INC.:

KIMERAGEN, INC.:

By: \_\_\_\_\_

Name:

Title:

Date:

By: \_\_\_\_\_

Name:

Title:

Date:

I agree to and accept the terms and conditions of this Agreement and agree to act as Investigators for the Research Programs established hereunder.

INVESTIGATORS:

INVESTIGATORS:

By: \_\_\_\_\_

Name: Dr. Hugh Mason

Address: \_\_\_\_\_

\_\_\_\_\_

Date: \_\_\_\_\_

By: \_\_\_\_\_

Name: Dr. Greg May

Address: \_\_\_\_\_

\_\_\_\_\_

Date: \_\_\_\_\_

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## SCHEDULE 1

Research Program

1. See Attachment A.
2. The Investigators shall be available from time to time, subject to reasonable prior notice being received from the Company to discuss and/or make presentations with respect to the performance of the Research Program set out in paragraph 1, above (including without limitation research protocols and methodology used in laboratory work conducted) and the research results generated in performance of the Research Program with representatives of the Company and such other persons as the Company may nominate from time to time, including without limitation potential investors and/or underwriters, the Company's auditors, the Company's patent counsel, media representatives and potential commercial partners and/or customers of the Company.
3. Investigators shall perform such other services relating to the subject matter of the Research Programs and assist the Company with such other matters relating to the subject matter of the Research Programs as are reasonably designated by the Company from time to time during the term of this Agreement.

Budget

See Attachment A.

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## KIRKLAND & ELLIS

PARTNERSHIPS INCLUDING PROFESSIONAL CORPORATIONS

Stephen P. H. Johnson  
To Call Writer Direct:  
212 446-4920

Citicorp Center  
153 East 53rd Street  
New York, New York 10022-4675  
212 446-4800

Facsimile:  
212 446-4900

December 5, 1996

### BY FEDERAL EXPRESS

Dr. Charles Arntzen  
Boyce Thompson Institute of Plant Research, Inc.  
Tower Road  
Cornell University  
Ithaca, New York 14853-1801

Re: Kimeragen

Dear Charlie:

It was a pleasure to meet you at the retreat in Maine.

I enclose four execution copies of the Research Collaboration Agreement. Please will you fill in the date of the MTA in paragraph 8(b). In addition, you and Kimeragen need to fill out the terms of Attachment A.

After signing the agreement, please have the agreements sent to Ramesh Kumar so that they may be executed by Kimeragen.

Please do not hesitate to call if you have any questions.

Yours sincerely,



Stephen P. H. Johnson

SPHJ:dp  
enclosure

cc: Ramesh Kumar, Ph.D.

001343

**KIMERAGEN, INC.****RESEARCH COLLABORATION AGREEMENT**

This Research Collaboration Agreement ("Agreement") is made by and between Kimeragen, Inc., a Delaware corporation, having an address at 300 Pheasant Run, Newtown, PA 18940 (the "Company") and the Boyce Thompson Institute of Plant Research, Inc. of Tower Road, Cornell University, Ithaca, New York 14853-1801 (the "Institute") effective as of October \_\_, 1996 (the "Effective Date").

WHEREAS, the Company and the Institute have agreed that the Institute will conduct research for, and engage in collaborative research with, the Company under the direction of Drs. Hugh Mason and Greg May, as joint principal investigators (the "Investigators") on the terms set forth in this Agreement.

NOW, THEREFORE, in consideration of the mutual covenants hereinafter set out and for good and valuable consideration, the receipt and sufficiency of which is hereby acknowledged, the parties agree as follows:

**1. RESEARCH**

(a) The Institute shall perform the research program set forth in Schedule 1 hereto and each such other research program(s) and/or modification(s) thereof, a written description of which is marked with a statement signed by the parties hereto as a research program for the purposes of this Agreement (each a "Research Program"), under the direction of the Investigators, for and in collaboration with the Company in accordance with the terms and conditions of this Agreement. Each Research Program shall include the agreed term of, and budget for, that Research Program. The Institute shall ensure that the highest standards are observed in conducting each Research Program.

(b) The Institute shall keep the Company advised as to the progress in performing each Research Program (including by making prompt written disclosure of all Research Results (as defined in Clause 5(a), below) and the Institute will, as requested by the Company, prepare any additional written reports with respect thereto. The Institute shall prepare a final report for each Research Program within sixty (60) days of completion of the Research Program. Subject to Clause 3, reports prepared for the Company shall be the sole property of the Company.

**2. COMPENSATION FOR RESEARCH COSTS**

The Company shall reimburse the Institute for its reasonable costs incurred in conduct of Research Programs in accordance with the budget agreed between the parties for the Research Program in each case. Unless otherwise agreed in the budget, payments from the Company shall not be due more frequently than quarterly. The Institute shall apply all payments received from the Company in accordance with the agreed budget for the applicable Research Program. Any funds paid by the Company and not used by the



Institute in the Research Program for which they were paid in any contract year shall be applied against payments due by the Company for that Research Program in the subsequent contract year, or, if that Research Program has been completed or terminated, shall be refunded to the Company or applied against amounts due by the Company to the Institute for any other current Research Program, at the Company's option.

### 3. PUBLICATION

The Investigators and other employees or contractors of the Institute that are staffed on any Research Program shall be entitled to publish or disclose research results or other material generated in performance of that Research Program, provided that the Institute shall: (1) provide a draft of the disclosure to the Company at least thirty (30) days in advance of submitting such disclosure for proposed publication or presentation; and (2) acknowledge the Company in the publication or disclosure as the collaborator in the Research Program. The Institute shall cause its employees and its contractors to make all reasonable changes requested by the Company. If the Company notifies the Institute that it considers that a proposed publication or presentation includes material which may be patentable, then the Institute shall cause its employees and its contractors to delay the proposed publication or presentation for at least an additional sixty (60) days or such longer period as agreed upon by the parties to permit a patent application with respect to that subject matter to be prepared and filed.

### 4. CONFIDENTIALITY AND MATERIAL TRANSFER

(a) In performing or otherwise in connection with each Research Program, (i) the Institute may acquire, receive, observe or generate, alone or jointly with others, information and/or material (including certain Biological Material as defined in this Clause 4) that is confidential or proprietary information of the Company ("Company Proprietary Information"); (ii) the Company may acquire, receive or observe information and/or material that is confidential or proprietary to the Institute ("Institute Proprietary Information"); and (iii) confidential information and/or material may be generated by persons carrying out the Research Program ("Research Information"). Subject to Clause 3, the Institute agrees not to disclose any Company Proprietary Information or Research Information to any third party or to use any Company Proprietary Information for any purpose other than performance of Research Programs pursuant to this Agreement, without prior written consent of the Company. The Company agrees not to disclose Institute Proprietary Information or Research Information to any third party without the Institute's written consent, provided, however, that the Company may disclose Research Information for any business purposes, including without limitation, for the purposes of developing and/or commercializing any technology or other subject matter assigned or licensed to it under this agreement or any other agreement between the parties. The Company Proprietary Information, Institute Proprietary Information and Research Information shall be referred to collectively as "Proprietary Information."

(b) Proprietary Information does not include information which: (i) is or later becomes available to the public through no breach of this Agreement; (ii) is obtained by the receiving party from a third party who had the legal right to possess and disclose the

information to that party and is not associated with the Research Program to which the Proprietary Information relates; (iii) the receiving party can show was already in its possession prior to (direct or indirect) receipt by the receiving party from, or disclosure to the receiving party by, the disclosing party; or (iv) is required to be disclosed under any applicable law or court order provided that the receiving party provides the disclosing party with prior written notice of the requirement for disclosure detailing the Proprietary Information to be disclosed, to enable the disclosing party to seek a protective order or otherwise prevent disclosure of such information.

(c) In the course of performing Research Programs hereunder, the Institute may receive from the Company biological material, (including without limitation cell cultures, organisms, proteins, nucleic acids, and/or other chemical or biochemical materials) ("Biological Material"). The Institute shall use the Biological Material only for the purposes of performing the Research Program in connection with which the Biological Material was provided, and shall not provide any Biological Material to any third party unless authorized in writing by the Company to do so, and then only in accordance with the Company's procedures for transfer of such Biological Material. All progeny of, modifications to, material derived or produced from, originating from, made using or which incorporate any element of any Biological Material or progeny thereof, or otherwise made in performance of any Research Program shall be included within the definition of Biological Material for the purposes of this Clause 4. The Biological Material is proprietary to the Company and shall remain the property of the Company notwithstanding that the Biological Material may be or come into the possession of the Institute and/or Investigators, and shall not be removed from the location designated by the Company for performance of the Research Program in connection with which the Biological Material was provided, without the Company's prior written consent.

(d) Following the termination of this Agreement or upon earlier request by the Company, the Institute shall promptly deliver all materials comprising or including any Company Proprietary Information including but not limited to, all written materials, computer media and Biological Material in its possession; and shall delete all Company Proprietary Information from computer disks or electronic storage facilities owned or used by the Institute and/or its employees and/or contractors.

## 5. OWNERSHIP OF INTELLECTUAL PROPERTY

(a) Subject to paragraph (b), below, all rights in and title to any and all inventions (whether patentable or non-patentable), know-how, trade secrets, tangible research property (including biological material), technical data and research results generated or otherwise gained from any Research Program (collectively "Research Results") and, to the extent applicable, any patent applications or patents covering any Research Results ("Research Patents") conceived or developed or applied for in the course of, or as the result of, conducting any Research Program shall be the property of the Company. The Company shall be responsible, at its cost, for the preparation, filing, prosecution and maintenance of any Research Patents. The Institute shall disclose all Research Results to the Company promptly in writing as provided in Clause 1(b).

(b) The rights granted to the Company under Clause 5(a) shall be subject to any prior rights which the U.S. Government or any agency thereof may have in and to Research patents as a result of 35 U.S.C. § 200 *et seq.* and 37 C.F.R. § 401 or otherwise. The Institute shall elect title to any Research Patent to which this Clause 5(b) applies pursuant to 35 U.S.C. § 202.

(c) The Institute hereby assigns to the Company all rights and title it may have to any Research Results and/or Research Patents and undertakes that it shall, and shall cause its employees and contractors (as required) to take all steps and actions and execute and record all documents necessary to record the assignment of the Research Results (and Research Patents, if any) and otherwise give effect to this Clause 5. Without limiting the foregoing, the Institute shall and shall cause its employees and contractors to provide all assistance reasonably required by the Company to give effect to this Clause 5 and enable the Company to prosecute and maintain Research Patents.

(d) The Company hereby grants the Institute a perpetual, royalty-free, worldwide license to practice under Research Patents for its own non-commercial research purposes. In consideration for that license, the Institute hereby grants the Company an exclusive option to any and all inventions (whether patentable or non-patentable), know-how, trade secrets, tangible research property (including biological material), technical data and research results generated or otherwise gained from any such licensed research and owned by the Institute (collectively "Licensed Research Results"), and, to the extent applicable, all patent applications and patents covering any such Licensed Research Results. The option granted herein shall extend for twenty-four (24) months from the Company being notified by the Institute of such Licensed Research Results. The option granted hereunder shall be exercisable at any time, or from time to time, during that option period by notice to the Institute in writing. Following exercise of the option by the Company such Licensed Research Results shall be treated as part of the Research Results and such patents or patent applications covering such Licensed Research Results shall be treated as Research Patents, and accordingly the consideration for the exercise of that option shall be as set out in Clause 6. Following exercise of the option, the parties shall take all steps and execute and record all documents necessary to assign the relevant Research Results and otherwise give effect to this Clause 5(d).

## 6. ROYALTY OBLIGATIONS

(a) As consideration for the rights granted to the Company under Clause 5, the Company hereby agrees to pay the Institute royalties as follows:

(i) The Company shall pay to the Institute a royalty of one and one-half percent (1½%) of the Net Sales (as defined below) of products that are covered by a valid claim of a Research Patent ("Products") up to a cumulative maximum royalty base of three hundred million dollars (U.S. \$300,000,000) in Net Sales, provided that in the event that any Products are subject to royalties under license and other agreements with third parties, the royalty payable to the Institute with respect to such Products shall be reduced so that the total royalty burden on such Products shall not exceed one and one-half percent (1½%), provided further that the royalty

payable to the Institute shall in no event be less than one quarter of one percent (¼%).

(ii) Royalties shall be payable on a country by country basis on sales of Products (other than sales by the Company to its Subsidiaries or licensees) where the Products were made, used or sold in any country in which there is a valid claim of a Research Patent which covers the Products in question, until the expiration of the last valid claim of a Research Patent in any country in which any Product is made, used or sold.

(iii) The Company shall pay to the Institute a percentage of any revenues received from third party licensees equal to ten percent (10%) of revenue payable by such licensees by reference to a valid claim of a Research Patent, subject to reduction as provided in Clause 6(a)(i) hereof, but in no event less than two and one half (2½%).

(b) Provided that Products are developed that are protected by a valid U.S. patent claim under a Research Patent, the Company shall make payments to the Institute at the beginning of each year following the issuance of the first U.S. Research Patent, which shall be creditable in full against running royalties. Such fees shall be as follows:

(i) Five thousand U.S. dollars (US\$5,000) upon issuance of the first U.S. Research Patent; and

(ii) The greater of (x) five thousand U.S. dollars (US\$5,000); and (y) twenty-five percent (25%) of the total payments made by Kimeragen to the Institute under this clause 6 in the preceding year; on or before each January 31 of each year thereafter prior to the expiration of the last valid claim of a Research Patent in any country in which any Product is made, used or sold.

(c) Royalties shall be payable by the Company annually in U.S. dollars (net of withholdings and other taxes) within sixty (60) days of the Company's fiscal year end (December 31) after the first commercial sale of any Product. Each royalty payment shall be accompanied by a royalty statement setting out how the royalties were calculated in accordance with this Clause 6. For the purposes of calculation of royalties, payments received from third parties shall be converted into U.S. Dollars according to the exchange rate quoted by the Wall Street Journal effective on the last day of the year to which the payments relate.

(d) For the purposes of this Agreement, "Subsidiary" shall mean, with respect to any person, any other person that, directly or indirectly, controls or is controlled by or is under common control with that first mentioned person. For the purposes of this Clause 6, (i) "Net Sales" shall mean the invoice price ex-works of Products sold by the Company or its Subsidiaries to a customer other than the Company and its Subsidiaries, less any discount, freight, taxes, duties, tariffs, fees and other charges, and after deduction of amounts credited to customers or written off as bad debts; (ii) where any Research Patent

is abandoned, expired or held invalid, it shall be treated as if no patent had been issued with respect to that Research Patent.

(e) The Company shall keep proper records of (i) all sales of Products made by the Company; (ii) all sales of Products, all invoices for such sales, and other information necessary for the calculation of Net Sales of Products; (iii) all licenses granted under or with respect to any Research Patents; and (iv) all payments received from third parties under or in respect of any such license. Subject to reasonable prior written notice, the Company shall allow the Institute access to such records during customary business hours for the purposes of verification of royalties paid and payable under this Agreement, provided that the Institute may not have access to such records more than once in any calendar year.

## 7. TERMINATION

(a) The term of this Agreement shall run from the Effective Date until December 31, 1997 or such later date as agreed upon by the parties in writing, provided that either the Institute or the Company may terminate this Agreement by sixty (60) days written notice to the other for any reason or for no reason.

(b) The Institute may terminate this Agreement by fourteen (14) days written notice if the Company commits any material breach of this agreement. The Company may terminate this Agreement by fourteen (14) days written notice if the Institute commits any material breach of this agreement or if the Investigators resigns from the Institute or becomes unwilling or unable to conduct any Research Program.

(c) Termination or expiration of this Agreement shall not affect: (i) the Company's obligation to pay for research performed under this Agreement in accordance with Clause 2; or (ii) the parties' obligations under Clauses 3 and 4, 5 and 6, above.

## 8. MISCELLANEOUS

(a) This Agreement shall be deemed to be a contract made under the law of the State of New York applicable to contracts made and performed entirely within the State, and for all purposes this Agreement shall be construed and interpreted in accordance with and be governed by the law of the State of New York.

(b) This Agreement may not be and shall not be deemed or construed to have been modified, amended, rescinded, canceled or waived in whole or in part, except by written instrument signed by the parties. This Agreement, including the Schedules attached hereto, constitutes and expresses the entire agreement and understanding between the parties with respect to its subject matter. Without limiting the foregoing, this Agreement supersedes the material transfer agreement between the parties dated October \_\_, 1996 and all activities conducted under that material transfer agreement shall be considered activities under the initial Research Program for the purposes of this Agreement.

(c) The Institute is an independent contractor and not an agent or partner of the Company. The Institute shall not enter into any agreement or incur any obligations on the Company's behalf, or commit the Company in any manner without the Company's prior consent.

(d) The Institute shall not assign or transfer its obligations under this Agreement without the prior written consent of the Company.

(e) Should any provision of this Agreement be illegal, invalid or unenforceable, all other terms and conditions of this Agreement shall remain in full force and effect.

IN WITNESS WHEREOF, the parties have executed this Agreement as of the date first set forth above.

BOYCE THOMPSON INSTITUTE OF  
PLANT RESEARCH, INC.:

KIMERAGEN, INC.:

By: \_\_\_\_\_

Name: \_\_\_\_\_  
Title: \_\_\_\_\_  
Date: \_\_\_\_\_

By: \_\_\_\_\_

Name: \_\_\_\_\_  
Title: \_\_\_\_\_  
Date: \_\_\_\_\_

I agree to and accept the terms and conditions of this Agreement and agree to act as Investigators for the Research Programs established hereunder.

INVESTIGATORS:

INVESTIGATORS:

By: \_\_\_\_\_

Name: Dr. Hugh Mason

Address: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Date: \_\_\_\_\_

By: \_\_\_\_\_

Name: Dr. Greg May

Address: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Date: \_\_\_\_\_

001351

## SCHEDULE 1

### Research Program

1. See Attachment A.
2. The Investigators shall be available from time to time, subject to reasonable prior notice being received from the Company to discuss and/or make presentations with respect to the performance of the Research Program set out in paragraph 1, above (including without limitation research protocols and methodology used in laboratory work conducted) and the research results generated in performance of the Research Program with representatives of the Company and such other persons as the Company may nominate from time to time, including without limitation potential investors and/or underwriters, the Company's auditors, the Company's patent counsel, media representatives and potential commercial partners and/or customers of the Company.
3. Investigators shall perform such other services relating to the subject matter of the Research Programs and assist the Company with such other matters relating to the subject matter of the Research Programs as are reasonably designated by the Company from time to time during the term of this Agreement.

### Budget

See Attachment A.

001352



10-21-96

I gene cleaned the frago- eluted in 20  $\mu$ L  $dH_2O$ .

set up ligation: 1  $\mu$ L vector  
1  $\mu$ L insert  $\rightarrow$  10 $\times$  at RT.

Completed genomic DNA isol.

- visible ppt in alcohol.
- spin, air dried  $\sim$  30 min.
- resusp 250  $\mu$ L  $dH_2O$ ; did not go into soln.
- I stored these samples at 4 $^{\circ}$ C.

Kipp

10-22-96

Chimeric oligo - Spin oligo 30' at RT, very visible yellowish color. Washed w/ 100  $\mu$ L -20 $^{\circ}$ C 70% EtOH, no spin. air dried  $\sim$  40 min.

resusp in 125  $\mu$ L  $dH_2O$  - 200 ng/ $\mu$ L.

The sample did not resusp very well.

I vtrd it for  $\sim$  5 min; there were still visible yellow chunks.

I added an add'l 125  $\mu$ L of  $dH_2O$ .

- still, it did not go into soln after vtr.

I heated the sample to 55 $^{\circ}$ C.

Today's Expts: Does the oligo bind to the gold beads?

Does the oligo fluoresce by itself in  $dH_2O$ ?

Can NT-1 protoplasts be electroporated to cause uptake of the oligo, and will the oligo fluoresce in NT-1 cells?

Can the oligo be introduced by PEG mediated protoplast transformation?

002188

10-27-96

### Preparation of gold -

100mg/ml sol'n  $\rightarrow$  5mg gold + 5ml  $\text{dH}_2\text{O}$ .

vtx, incubate on ice.

### Protoplasting -

Used 40ml cells from 10 fl (4  $\rightarrow$  40).

Began enzyme mixing  $\rightarrow$  75 mls enzyme, according to protocol.

The settled volume of the cells is  $\sim$  8ml.

I added 7ml of cells from an older culture to increase the # of cells slightly.

Spin enzyme mix in 30ml Corex tubes 10' at 10,000.

Washed cells 2x in PAM.

Added 45ml enzyme sol'n to  $\sim$  9ml cells.

Plated in 3 plates, began shaking at 6:15 PM, dark.

Did not save cond media, will look at cells directly.

Gold particles - took out 1  $\mu\text{L}$  - untreated

Took remaining 8  $\mu\text{L}$ , added 1  $\mu\text{L}$  oligo (100ng), 4  $\mu\text{L}$  2.5M  $\text{CaCl}_2$  and 1.85  $\mu\text{L}$  spermidine.

vtx

on ice  $\sim$  30 min (calls for 10 min).

washed particles w/ 100  $\mu\text{L}$   $\text{dH}_2\text{O}$ .

Looked at them under the scope. Fairly uniform size.

No fluorescence of beads  $\rightarrow$  oligo does not stick to particles, at least at this [I] and cond.

002189

10-22-96

## NT-1 protoplast - PEG transformation

## Stable transformation of protoplasts of SR1 tobacco without electroporation

Several methods have been published for transformation of protoplasts without the use of electroporation (e.g. 4-6)]. The majority of these methods result in a transformation frequency lower than that for the method presented above but easily produce enough transformants for the majority of cases where one wants to introduce foreign DNA into a plant cell. Here we present a simple method which, for SR1 protoplasts, gives a transformation frequency comparable to that with electroporation [17].

## Steps in the procedure

1. Prepare the protoplasts as in the first protocol as far as the end of step 5. Instead of washing by further flotation in K3 medium wash the protoplasts two times by resuspension in W5 solution and centrifugation at 600 rpm for 5 min, followed by counting.
2. Resuspend the protoplasts at a density of  $1.6 \times 10^6$ /ml in the mannitol/magnesium solution. Heat shock for 5 min at  $45^\circ\text{C}$  followed by cooling to room temperature and distribute 0.3-ml aliquots into 5-ml sterile plastic tubes.
3. Add 30  $\mu\text{l}$  of the DNA solution and mix, followed by 300  $\mu\text{l}$  of the PEG solution. Incubate for 25-30 min at room temperature with occasional shaking.
4. Gradually add 10 ml of W5 solution over about 10 min and then centrifuge for 10 min at 600 rpm.
5. Resuspend the protoplasts in 1 ml of K3 medium, transfer to a 9-cm petri dish and add 7 ml of a 1:1 mixture of K3 and H media containing 0.6% w/v SeaPlaque agarose. Mix the protoplasts well but gently with the agarose medium, and allow this to set. Do not disturb the dishes until the medium is solid, as this will cause damage to the protoplasts.

## Notes

1. Before using the protoplasts for transformation they should have been in W5 for at least 30 min. If they are not used immediately they can be stored at  $8^\circ\text{C}$  in W5 for up to 8 h without loss of competence for transformation.
2. As little as possible W5 should be transferred into the mannitol solution. Transformation should take place without delay after transfer to this solution. The exact concentration of magnesium appears to be important for the transformation frequency. This should be checked, in the range 5-50 mM, especially for protoplasts other than SR1.
3. The volumes here can be altered according to the experiment. The concentration of PEG appears to be important and should be optimized for each system. Also, the molecular weight of the PEG used can be important. For more fragile protoplasts PEG 6000 may be

better. The concentration of DNA (ca. 10  $\mu\text{g}/\text{ml}$  pABD1, 50  $\mu\text{g}/\text{ml}$  carrier) should be worked out on the basis of the total volume of the transformation mix after addition of the PEG.

4. For example, 1 ml, 2 ml, 7 ml at 3-min intervals. This step is not essential for SR1 protoplasts but improves the survival and is essential for other less stable protoplasts.

## Solutions (sterile)

In general all solutions and media are sterilized by filtration through 22- $\mu\text{m}$  filters. Some simple salt solutions can be sterilized by autoclaving.

- 0.5 M mannitol containing 15 mM  $\text{MgCl}_2$ , 0.1% MES, pH 5.6 with KOH
- DNA solution: 0.3 mg/ml pABD1 linearized with *Sma*I and 1 mg/ml of calf thymus DNA (Sigma) in double-distilled water. The DNA is sterilized by precipitation in 70% ethanol followed by a wash in 70% ethanol and drying in a sterile flow hood. Calf thymus DNA is sheared by passage through an 18-G needle to an average size of 5-10 kb.
- PEG solution: 40% w/v PEG 4000 (Merck) in 0.4 M mannitol, 0.1 M  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , pH 8 with KOH. The PEG is dissolved in 0.4 M mannitol, 0.1 M  $\text{Ca}(\text{NO}_3)_2$  (i.e. the final concentration of these two components will be lower due to the volume of the PEG). The pH takes at least 2-3 h to stabilize in this solution
- W5 solution [20]: 154 mM NaCl, 125 mM  $\text{CaCl}_2$ , 5 mM KCl, 5 mM glucose, pH 6.0 with KOH
- K3/H (1:1 mix, Table 1) medium, liquid
- K3/H (1:1 mix, Table 1) medium containing 0.6% SeaPlaque agarose (agarose sterilized dry, then K3 medium added and melted, followed by H medium)

more fragile  
pH 11.5  
PEG 6000/4000

## mannitol

50 ml: 25 ml 1M mannitol

0.375 ml 1M  $\text{MgCl}_2$

1 ml 100 mM MES

25 ml 1M mannitol

0.375 ml 2M  $\text{MgCl}_2$

1.100 ml 100 mM MES

pH 5.6

## PEG

50 ml: 20 g PEG 3350

20 ml 1M mannitol

2 ml 2.5M  $\text{Ca}(\text{NO}_3)_2$

20 g PEG 3350

20 ml 1M mannitol

2 ml 2.5M  $\text{Ca}(\text{NO}_3)_2$

## W5

20 ml

10 ml

0.5 ml

0.454 ml

7.7 ml

10 ml

0.5 ml

0.454 ml

pH 6.0

7.7 ml 4M NaCl

10 ml 25M  $\text{CaCl}_2$

0.5 ml KCl 2M

0.454 ml 40% glucose

7.7 ml 4M NaCl

10 ml 25M  $\text{CaCl}_2$

0.5 ml 2M KCl

0.454 ml 40% glucose

pH 6.0

made: Mannitol/Mg media

50

PEG media  $\rightarrow$  very difficult to pH!

25

filter after.

W5 media

200

The pH of the PEG media will not stabilize. I added 1 drop of 0.2M HCl and the pH went from 12  $\rightarrow$  2!

9:15 PM - checked protoplasts - some present, lots of clumps however, many of the clumps appear semi-digested

Continued - incubation until 9:45.

002190

Two of the three plates have lots of chunks. I filtered the cells from these through a 140  $\mu\text{m}$  filter cap.

10-22-96

I pooled the cells from the third plate and the  
 flow through from the filtration.  
 - a large amount of stuff was caught on the filter  
 I think that it was a good idea.

I washed the protopls. in PIM 2x.

Resusp. in 20 mL PDR.

Hemocytometer - counted ~ 100 cells total, 2 grids

$$50 (10^4) = 0.5 \times 10^6$$

I will use  $1 \times 10^6$  cells / electroporation

Spin cells resuspended in 10 mL PDR

On ice ~ 10 min.

Elpns: Control - no DNA  
 $0.500 \mu\text{g} \rightarrow 5 \mu\text{L}$  at  $100 \mu\text{g}/\mu\text{L}$   
 $1.5 \mu\text{g} \quad 15 \mu\text{L}$   
 $3 \mu\text{g} \quad 30 \mu\text{L}$

Elpn at  $\lambda/250$ , volt setting 0.75 inverted to mix.

$t_c = 5'$  Very low - wrong parameters?  
 no, see pg 64.

$t_c$  difference may be due to cell density

Inverted several times to mix

Set up slides -

pipetted ~ 50  $\mu\text{L}$  (blue hp).

Two spots per slide - Fluorescence microscope

10-22-96

I looked at a few of the leftmost unexpt. prots.

They have some faint green background fluor.  
It seems that some cell wall component fluoresces  
because I cannot see any fluor. of individual  
cells - the background is only seen on cell clumps.

Control - Very much the same as unexpt cells.

On visible, some lysed cells are seen. These  
have no fluorescence.

Samples - I was able to find several cells  
that were glowily brightly green. The fluorescence  
is much more intense and a brighter more neon  
colored fluorescence.

500ng - I can see a variety of cells w/ F.  
single cells + 1 -  
whole clumps + 1 -  
partially F clumps

1.5mg - The % of cell clumps that show F is  
higher.

3mg The % is again higher. Also I notice that  
the F. is localized in the nucleus of the cells.

The slides I looked at earlier showed F. throughout.  
These cells show a dramatic [ ] of the F. in  
the nucleus.

I have been photographing specific samples as I  
go, using 100 speed film on the "1" setting.

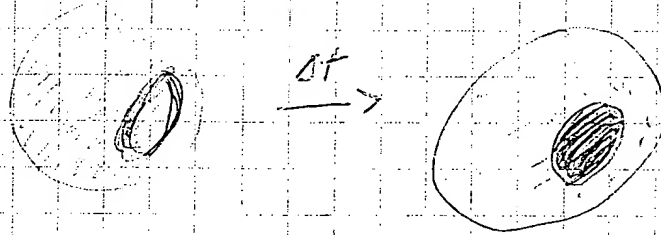
In each case, I take a picture of the cells  
in visible light, then under the UV.

002192

10-22-96

In order to see the F, it is critical to turn off the VIS hv source. However, for very intense samples, faint blue color is visible w/ both lights on.

I am curious if I just witnessed a time course of the oligo migrating into the nucleus. The first slides had uniform F, while the later cells had very concentrated F in the nucleus.



1 Epid 2  $\mu$ L of the 210.1+gfp ligation into DH5+  
to 4.5

I inoculated a fresh 40 mL of N1-1 cells for Friday.  
Plated CB9 after recovery, 12:30 AM.

happy.

10-23-96

Greg will develop the film of last night's photos and make slides.

There are no colonies yet.

TA-

PCR screen - >50 colonies

Selected 12

TEV/GFP-iso

begin ~6 PM

92 45

no control.

40 45 x 25

72 40

Finished 7:45 PM.

002193

10-23-96

The film showed no F. photos!

I set up some new slides. The cells have been stored in PCR O/N in ep. tubes. The slides from yesterday are dried.

Picked up in version - new slides

Spoke to G. Blaisdell -

F. photos should have  $> 1$  min exposure. Gary helped me set up.

Camera set up on "B". I will use Gary's clock - can set it up to hold, then disconnect.

VIS photos on setting 1 on 2 - F. on "B"

There is some F. in the cells, but the F. is less intense and more diffuse. The background is higher - presumably the cell walls are regenerating.

Began the gel of the PCR products -

I took a roll of film using 1-3 min exposures

single cells

some nuclear films.

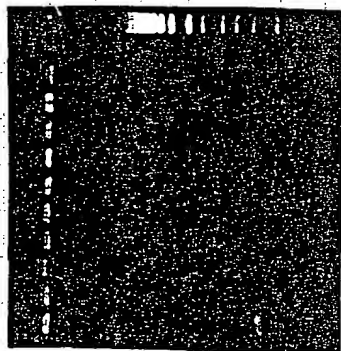
clumps +/-

partially HF clumps

I took some VIS pictures as well

I took some UV pics of background F.

PCR gel



picked

#1/2

into LBA

at 37°C

Kyp

002194



11-21-96



210.1 RI, NcoI 2-part

↑  
undigested

↓  
digested vectors

1 prep-a-gened, resusp 20  $\mu$ l  $H_2O$

1 set up a ligation w/ the gfp nos frag. at RT.

ALS primers -

5' #1 <sup>XX</sup> GGGGTACC GGATTTC CCGCGTTTG

hits frame  
shift

KpnI

region of homology

5' #2 <sup>XX</sup> GGGGTACC AGCGGCTCGCTGACGG

KpnI

region of homology

hits heterologous  
region

5' 3' #1 CGGGATCCGAACACCCACCCCCAC

BamHI

region of homology

Oligo design based upon Bedbrook EMBO J 7(5) p1246

5' primer 1 against frame shift in class I vs II

5' primer 2 against region of heterogeneity I v. II

3' primer 3 against shared region

002214



11-21-96

Bed Brook:

102 L V E A I E R E G V T D V F A Y P G G A S M E I H Q I A L T H S S I I R N V I P R  
 304 C T G T G G A G G C T C T G A A G A G A G G G T T A C G G A C G T C T T T G C G T A C C A G G T G C C G T T C C A T G G A G A T T C A C C A A G C T T T G A C C G G T T C A A G C A T C A T C G C A A C G G C T G C C A C G T  
 .....C.....T.....C.....G.....A.....C.....  
 142 H E O G G V F A A E G Y A R A T G F P P G V C I A T S G P G A T N L V S G L A D A  
 424 C A C G A G C A G G C G G T G T T C G C G C T G A G G G T T A C G C A C G G C A C C G G A T T T C C G G C T T G C A T T G C C A C C T C T G G C C C G G C C A C C A A T C T G T C A G C G C C T C G C T G A C G G  
 .....T.....C.....C.....C.....T.....G.....C.....  
 182 L L D S V P I V A I T G O V P R R M I G T D A F O E T P I V E V T H S I T K H N  
 545 C T A C T G G A T A G C G T C C C A T T G T G C T A T A C A G G T C A A G T G C A G T A G G T A C T G A T G C T T T C A G G A A C T C C A T T G T T G A G G T A A C T A G A T C G A T T A C C A A G C A A T  
 .....C.....C.....G.....  
 222 Y L V M D V E D I P R V V R E A F F L A R S G R P G P V L I D V P K D I Q O O L  
 665 T A T C T G T T A T G G A C G T A G A G A T T C C T A G G G T T G T A C G T G A A G C T T T T T C C G G A G A T C G G G C G G C C T G G C C C T A T T T T G A T G A T G A C T A A G G A T A T T C A G C A A C A A T  
 .....G.....T.....G.....  
 262 V I P D W D O P M R L P G Y H S R L P K L P N E M L L E O I V R L I S E S K K P  
 785 G T G A T A C C T G A C T G G G A T C A G C A A T G A G G T T A C C T A A A T T G C C A A T G A G A T G C T T T T A G A A C A A T T G T T A G G C T T A T T C T G A G T C A A G A A G C C  
 .....G.....  
 302 V L Y I V G G C S Q S S E D L R R F V E L T G I P V A S T L M G L G A F P T G D  
 905 G T T T G T A T G T G G G G G T G G G T T C G C A A T C G A G T G A G G A C T T G A G A C G A T T C G T G A G C T C A C G G G T A C C C C G T G G C A A G T A C T T T G A T G G G T C T T G A G C T T T T C C A A C T G G G G T  
 .....G.....C.....E.....  
 342 E L S L S M L G M H G T V Y A N Y A V D S S D L L L A P G V R F D D R V T G K L  
 1025 G A G C T T T C C C T T T C A A T G T T G G G T A T G C A T G G T A C T G T T A T G C T A A T T A T G C T G T G A C A G T A G T A T T G T T G C T C C A T T T G G G G T G A G G T T T G A T G A G A G T T A C T G G A A G T T A  
 .....A.....

ALS  
 genomic  
 sequence  
 (no introns)

PCR product seq. and map (file)

Fpp

according to the sequence, MapDraw site analysis indicates  
 a Cla I site and a H3 site with the sequence -

therefore the Bam / Xpn sites in the primers will be effective.

Kpp

11-22-96

Transformed 2 µl ligation into DH5α.  
 plated LBA

Chimeric Oligo - ppt. onto Gold -

2 protocols - 1 for AU, 1 for Tungsten.  
 (NSH) (DH)

Ordered Tungsten (BioRad).

002215

11-22-96

Au ppt. - uses 2.5M  $\text{CaCl}_2$  and spermidine

3mg Au  $\rightarrow$  add 50  $\mu\text{L}$  100% EtOH.  
 $\rightarrow$  sonicate x3 (on ice).  
(1 fold, #1, 30 sec)

- wash  $\text{dH}_2\text{O}$ .
- resusp 50  $\mu\text{L}$   $\text{dH}_2\text{O}$

ppt.: 50  $\mu\text{L}$  Au resusp.  
5  $\mu\text{L}$  plasmid DNA (1  $\mu\text{g}/\mu\text{L}$ )  
50  $\mu\text{L}$  2.5M  $\text{CaCl}_2$   
20  $\mu\text{L}$  0.1M spermidine

I used 8  $\mu\text{L}$  of the dimeric oligo (800 ng).

I vtr gently as I added the components.

Fluorescence microscopy - I could not see any F.  
on the particles.

Kipp.

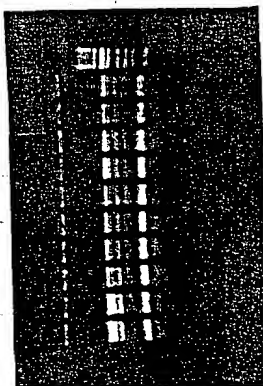
11-24-96

GFP-D10.1 >30 colonies.

12 for PCR screen - GFP / TEV

92 1  
42 1 x25  
72 1

gfp10.1  
putative  
PCR  
screen.



1-12 PCR on ligations

11-24-96

The Au ppt. fouled yesterday - it may be due to the older spermidine. Hugg's recommend fresh spermidine.

I ordered a new aliquot of spermidine (Sigma).

I inoculated #1 and #2 from CIP210.1 into LBt.

Hugg.

11-25-96

HM told me that He and Niga have determined that the TEV primer is contaminated w/ a LT plasmid, giving a spurious 500bp. band.

I plasmid prep'd #1 and #2, but did not digest.

Hugg.

11-27-96 break

The ACS primers are here. Resusp at 100  $\mu$ M in dH<sub>2</sub>O.  
Filed ppw. Spermidine arrived.

Hugg.

11-28-12-1 break

12-1-12-3 ill.

12-4-96

Biolistic particles - pilot exp.

I will use Tungsten, cheaper.

I am almost out of the last aliquot of the chimeric oligo.

I open out another 25  $\mu$ g aliquot, washed.

- added 50  $\mu$ g dH<sub>2</sub>O + 500 ng ppt.

- at 65°C.

002217

12-4-96

ALS genomic PCR - 4 rxns.

used NT genomic DNA (MT mix) - (see pg 101)

A260 = 0.658 took 120  $\mu$ L + 1  $\mu$ L RNase  
at 37°C 1 min

$\phi$ -OH/CHCl<sub>3</sub> (v/v)

used 30  $\mu$ L for each rxn.

Premix w/ primers 1/2: 100  $\mu$ L rxns.

45x	10 $\mu$ L	10x PCR (M <sub>g</sub> free)
	16 $\mu$ L	1.25 mM dNTPs
	6 $\mu$ L	25 mM MgCl <sub>2</sub>
	1 $\mu$ L	Taq
	1 $\mu$ L	primer 3
	34 $\mu$ L	dH <sub>2</sub> O

added primers 1 and 2 separately.

1-3 A	2-3 A	pm (no DNA control).
1-3 B	2-3 B	

began 3PM

92°C	1
58°C	45 x 25.
72°C	45

The chimeric oligo will not resusp. - froze -80°C.

Kyp.

12-5-96

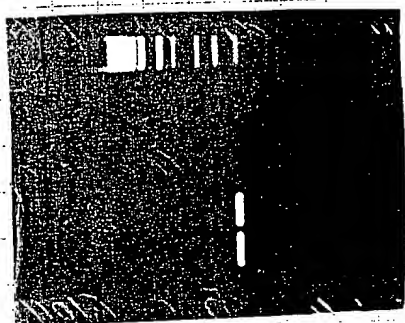
ALS PCR gel - ran 30  $\mu$ L aliquots:

PM 1-3A 1-3B 2-3A 2-3B  $\hookrightarrow$  Std

002218

12-5-96

ALS  
genomic  
PCR



2-3 B

2-3 A

1-3 B

1-3 A

Pr

2-3 failed

1-3 appropriate  
band

↑ major band ~ 500 bp  
minor band ~ 750 bp.

yield looks very good

1  $\text{CHCl}_3$  1-3A and 1-3B to remove  $\text{EtBr}$ , ppt  $\text{NaOAc}$  +  $\text{EtOH}$ .  
-  $80^\circ\text{C}$  30 min

### Microparticle Pilot Expts -

Prepared 1 mL Tungsten (60 ng)

I will use tRNA as a chimeric oligo mimic.

I took 3  $\mu\text{g}$  tRNA and kinased it:

3  $\mu\text{g}$  (3  $\mu\text{L}$ ) tRNA + : 6  $\mu\text{L}$  10x kinase buffer  
heated tRNA in  $\text{dH}_2\text{O}$  to 10  $\mu\text{L}$   $\gamma\text{-}^{32}\text{P}$  (old)  
90  $^\circ\text{C}$  4 min, quick cool down. 2  $\mu\text{L}$  kinase  
41.37  $\mu\text{L}$   $\text{dH}_2\text{O}$  → total = 62  $\mu\text{L}$ .  
at 37  $^\circ\text{C}$  for 15 min.

Run through spin column.  $\approx 1/2$  incorporated.

ppts: 5 - 5  $\mu\text{L}$  kinased tRNA + 25  $\mu\text{L}$  T + 25  $\text{CaCl}_2$  + 10  $\text{Spec}$ .

10 - 10  $\mu\text{L}$  tRNA + →

15 - 15  $\mu\text{L}$  tRNA + →

15+1 - 15  $\mu\text{L}$  tRNA + 1  $\mu\text{g}$  (1  $\mu\text{L}$ ) Salmon  
Spec DNA + →

15+2 - 15  $\mu\text{L}$  tRNA + 2  $\mu\text{g}$  (2  $\mu\text{L}$ ) SS DNA + →

002219

12-5-96

The tungsten protocol recommends vta followed by a 10 min incubation.

I saved the supernatant and the wash for each ppt. to assess the efficiency.

I resusp the final pellets in 35  $\mu$ l EtOH.

### Scintillation Counting -

Took 1  $\mu$ l of:

TRNA removed max

"TS"

"T10"

T15

T15+1

T15+2

TS & super

T10 "

T15 "

T15+1 "

T15+2 "

TS wash

T10 "

T15 "

T15+1 "

T15+2 "

Counted on  
channel 4.

Results (table on pg 120) - (Note - do not add to 100%)

Best cond - 15  $\mu$ l + 1  $\mu$ g carrier  $\rightarrow$   $\approx$  66% ppt to particles.

In general - more TRNA, more ppt.

- volume of TRNA added may not affect ppt

In more dilute samples 25  $\mu$ l  $\text{CaCl}_2$  + 10  $\mu$ l S  
was sufficient to achieve ppt.

carrier - too much reduced binding?

002220

12-5-96

1000 UL  
SAMP POS CH CPM 25% TIME ELTIME

1	267	1	8092.00	2.22	1.00	1.60	fRNA 1 $\mu$ l mix
		2	60703.00	.81			
2	268	1	185.00	14.70	1.00	2.74	T 5 1A
		2	1389.00	5.36			
3	269	1	628.00	7.62	1.00	3.98	T 10 "
		2	4403.00	3.01			
4	270	1	1923.00	4.56	1.00	5.02	T 15 "
		2	12251.00	1.80			
5	271	1	2329.00	4.14	1.00	6.16	T 15+1 "
		2	17246.00	1.52			
6	272	1	920.00	6.59	1.00	7.30	T 15+2 "
		2	5506.00	2.69			
7	273	1	127.00	17.74	1.00	8.44	wash 1 5
		2	2489.00	4.00			
8	274	1	171.00	15.28	1.00	9.58	wash 1 10
		2	3824.00	3.23			
9	275	1	236.00	13.01	1.00	10.72	15
		2	5273.00	2.75			
10	276	1	228.00	13.24	1.00	11.86	15+1
		2	5297.00	2.74			
11	277	1	319.00	11.19	1.00	13.00	15+2
		2	6392.00	2.50			

SAMP POS CH CPM 25% TIME ELTIME

12	278	1	25.00	40.00	1.00	14.27	wash 2 ↓
		2	45.00	29.77			
13	279	1	36.00	33.33	1.00	15.40	
		2	118.00	16.40			
14	280	1	37.00	32.86	1.00	16.54	
		2	78.00	22.64			
15	281	1	21.00	43.61	1.00	17.68	
		2	60.00	25.80			
16	282	1	18.00	47.11	1.00	18.82	
		2	38.00	32.42			

002221

12-5-96

tRNA → Tungsten ppt. Data.

Sheet1

sample	volume	counts	Total counts measured Vol x Counts	Total possible counts 60703 x Vol tRNA	% of Total
tRNA		60703			
T5	35	1389	48615	303515	16.02
T10	35	4403	154105	607030	25.39
T15	35	12251	428785	910545	47.09
T15,1	35	17246	603610	910545	66.29
T15,2	35	5506	87115	910545	9.57
Wash T5	60	2489	149340	303515	49.20
Wash T10	65	3824	248560	607030	40.95
Wash T15	70	5273	369110	910545	40.54
Wash T15,1	71	5297	376087	910545	41.30
Wash T15,2	72	6392	460224	910545	50.54

microg. tRNA

3 in 62	0.048
5 microliters	0.242
10	0.484
15	0.726

Washes not calculated, counts very low.

12-6-96.

Spun ALS PCR 1-3A/B. Resusp. dH<sub>2</sub>O.

Digested 1/2 of 1-3A w/ BamHI in buffer E.  
- began 10:15 AM.

Ø-CH/CHCl<sub>3</sub> xt. ppt w/ NaOAc + EtOH at -80°C 30 min.  
- resusp 10 µl dH<sub>2</sub>O  
- digested Kpn in buffer J.

after 75 min ran through spin column  
Ø-CH/CHCl<sub>3</sub> ppt again

Resusp 30 µl

Ligated 2.5 µl to 1 µl Bam/Kpn PKS vector.  
- 10 µl vol.

002222



12-6-96

ligated at RT.

Inoculated NT-1 cells  $4 \rightarrow 40$  for ppt? on Monday

also plated 6 plates of NT-1 cells on NT solid media

Kpp

12-7-96

TF ALS lig via Eppn.

plated  $\frac{1}{3}$  cells on LBA + X/I. at 37°C d.p.

Kpp.

12-8-96

Two whites > 15 blue.

plated R of cells on two LBAX1 plates, inoculated  
#1, 2 in LBA -

Kpp.

12-9-96

Moved ALS PCR #1/2 to 4°C.

Inoculated ALS PCR 3, 4 in LBA (only 2 more whites)

PPL - autoclaved feathers / pipets

prepared 50 mL enz. mix in P/M, according to the recipe.

ppt. 70 mL cells  $\rightarrow$  ~12 mL.

open enz. mix

PPL digestion began at ~~12~~ 1 PM.

Preparation of Tungsten w/ Chimeric oligo-

25  $\mu$ L Tung. + 10  $\mu$ L (1ng) oligo + 25  $\mu$ L 25M  $\text{CaCl}_2$  + 10  $\mu$ L spermidine

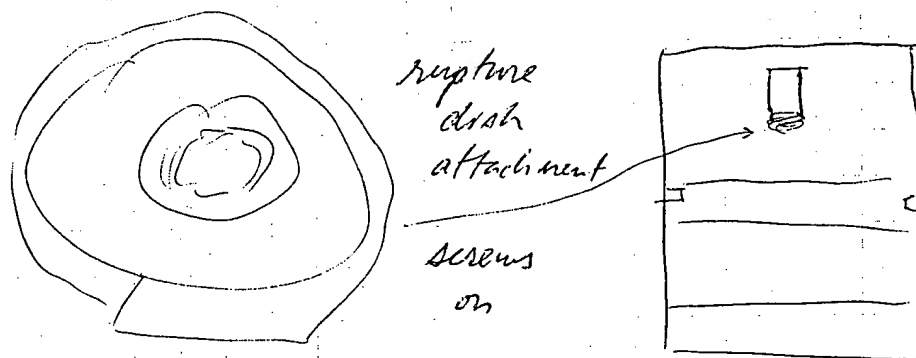
mixed, and allowed to ppt 10 min at RT.

002223

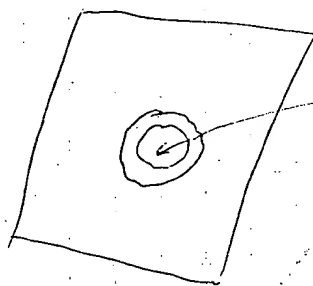
12-9-96

Am cut various tissues - flowers  
onion  
(NT-1)

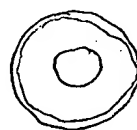
Biologic gun - 900 lb Hg (2 rupture disks) -



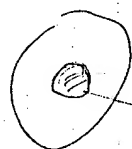
platform:



disposable  
wire mesh



flying dish  
holder (metal)



disk (plastic)

spot of sample

The plastic flying dish fits into the metal holder -

sample is added.

The platform is assembled: mesh inside, then a flying dish over it, inverted. The platform slides into the gun.

We used 2 rupture disks - the rupture disk piece screws securely onto the nozzle.

Each component is changed after each shot.

002224

12-9-96

Shot 3 controls - Tung only

Shot 12 samples -

By the time the shooting was complete, it was ~ 5 PM  
- The protoplasts were osmotically stressed  
- deformed shapes

- upon washing, the ppt's turned an odd color  
- I abandoned the ppt's for today.

Fluorescence microscopy -

I looked at the particles and all the samples.

The particles showed only a tiny amount of F<sub>i</sub> perhaps  
1:1000.

Not surprisingly, I did not see any fluorescence in the  
samples. No photos taken.

I will have to alter the ppt. cond of the oligo.

Kipp

12-10-96

I ppt'd the 4 ALS subclones via kit.

- 1.75 mL cells, resusp in 40  $\mu$ L.

- kept samples on ice.

I continued to try to resusp. the 2nd aliquot of the  
chimeric oligo, at 125 ng/ $\mu$ L in dH<sub>2</sub>O.

Microparticle ppt. of Oligo - GOLD.

I will use gold - less toxic, might as well practice on  
what we will use, regardless of cost.

~10 mg Gold, resusp 150  $\mu$ L 100% EtOH (Wash protocol)

002225

12-10-96

Sonicated 3x at 30 sec (Hold, duty constant, 1)

- volume only  $\sim 60 \mu\text{g}$

- splattering and evaporation of EtOH (? heat?)

assumed 6 mg left.

washed and resusp. by sonication-

### PPT COND

#1:  $10 \mu\text{L Au}$ , +  $10 \mu\text{L oligo}$  (500 ng) +  $100 \mu\text{L } 2.5\text{M CaCl}_2$  +  $40 \mu\text{L spermidine}$

if reagents  
old at  
addition

vtx during additions, and for 4 minutes afterward  
↳ at setting 1.

Fluorescence microscopy: large greenish crystals present, only  
perhaps 1/1000 particles coated.

#2.  $10 \mu\text{L Au}$ , +  $20 \mu\text{L oligo}$  (1 ng) +  $200 \mu\text{L } 2.5\text{M CaCl}_2$  +  $80 \mu\text{L spermidine}$

vtx during and for 8 min. subsequent..

Fluorescence: large green crystals, perhaps 1/1000 particles

#3.  $10 \mu\text{L Au}$  +  $30 \mu\text{L oligo}$  (1.5 ng) +  $180 \mu\text{L } 2.5\text{M CaCl}_2$  +  $40 \mu\text{L spermidine}$

vtx 2 min, add another  $40 \mu\text{L spermidine}$

vtx 8 additional minutes

wait 10' at RT.

Fluorescence: no crystals, but only  $\sim 1/1000$  particles

end aliquot  
to F-oligo

#4  $10 \mu\text{L Au}$  +  $20 \mu\text{L oligo}$  (<sup>1.2</sup> ~~1.2~~  $\mu\text{g}$ ) +  $25 \mu\text{L } 2.5\text{M CaCl}_2$  +  $10 \mu\text{L spermidine}$

vtx 10 min and during addition

wait 5 min

Fluorescence: The particles show a higher % of coating, perhaps

002226

12-10-96

This level of ppt. is still not sufficient to reliably shoot plant material. I will have to try to find better conditions.

It seems that less  $\text{CaCl}_2$  and spermidine volumes have worked better - no crystals - perhaps more DNA will enhance ppt.

Kyp

12-11-96

1 denatured 12  $\mu\text{L}$  (of 40) of each of the ALS PCR products.  
12 + 2  $\mu\text{L}$  2M NaOH / 2m EDTA + 6  $\mu\text{L}$   $\text{dH}_2\text{O}$ .

at 37°C, 30 min.

ppt. samples in NaOH + EDTA - brought volumes to 100  $\mu\text{L}$  w/  $\text{dH}_2\text{O}$ .  
- at -80°C.

Microparticle ppt. - sonicated particles to resuspend.

Repeated #4 (12-10)

10  $\mu\text{L}$  Au + 20  $\mu\text{L}$  oligo (1.2  $\mu\text{g}$ ) + 25  $\mu\text{L}$   $\text{CaCl}_2$  + 10  $\mu\text{L}$  spermidine

vtx 10 min after and while reagents added.  
wait 5 min, c'fuge, wash, resusp.

Fluorescence - Photography - ~ 1/200 particles covered  
took photos of particles on VIS, UV

#5 25  $\mu\text{L}$  Au + 40  $\mu\text{L}$  oligo (~2.5  $\mu\text{g}$ ) + 75  $\mu\text{L}$  2.5M  $\text{CaCl}_2$  + 30  $\mu\text{L}$  spermidine

vtx 10 min after and while reagents added  
wait 10 min

c'fuge 5 min at 12K. wash 100% ethanol, resusp. 50  $\mu\text{L}$  100% ethanol.

Fluorescence: Photography - seeming  $\frac{1}{2}$  - each particle  
has fluorescence on it. Took several photos.

002227

12-11-96

I prepared a second aliquot of particles in exactly the same way -

GM prepared tissues for shooting -  
3 potato slices  
3 onion slices  
2 pollen  
1 flower

Shooting at Biotek - ~1000 psi

2 rupture disks -

1 potato, 1 onion no DNA controls

others shot w/ DNA + F. (labelled DNA prep #1 or DNA prep #2)

Return to BT1 -

Fluorescence microscopy -

Protoplasts (I had prepared pp's this AM using 12 mL cells + 30 mL enzyme).

PEG method - according to protocol

10 mL oligo (0.0 mg)

30 mL oligo (1.2 mg)

30  $\mu$ L oligo (1.8 mg)

No oligo

300  $\mu$ L cells @  $1.8 \times 10^6$  / mL

Heat shock at 45°C 5 min

added DNAs + 300  $\mu$ L PEG reagent, mixed incubated at RT.

Elp'N - Elp 2 aliquots of cells.

The end result is that the pp's do not show any conclusive result because the background fluorescence is very high.

002228

12-11-96

microscopy of shot samples -

We saw various green spots imbedded w/in the potato tissues

We saw 2 clusters of onion cells that apparently have been treated w/ the drug. Took photos.

GM realized the camera wasn't set up properly, so the photos from this AM did not work.  
- must see target to photograph

Took 2 pictures of fluorescing particles  
- ~1-2 min exposures each

Took film to Fox Photo - will be ready tomorrow AM.

Left plasmids ppt'ing at  $-80^{\circ}\text{C}$ .

Kpp

12-12-96

Meeting w/ Kinnerager - photos turned out.

Ramesh Kumar, K's scientist.

Strand transfer assay

Rec 2 mediated process? How does Rec 2 work?

Kpp

002229

12-13-96

# ALS sequencing-

open plasmids, resuspended in 7  $\mu$ L dH<sub>2</sub>O  
+ 2  $\mu$ L 10x Run buffer  
+ 1  $\mu$ L primer (1  $\mu$ M) #1

at 37°C 60 minutes

set up termination mixes

set up sequencing premix:

1 $\mu$ L DTT 0.1M
0.5 $\mu$ L 35S
2 $\mu$ L labelling mix (1:5)

4.5 X 5

4.5  $\mu$ L  
9  $\mu$ L

5  $\mu$ L DTT  
2.5  $\mu$ L 35S  
10  $\mu$ L labelling mix (1:5)

initiated reactions by adding 2  $\mu$ L sequenase.

ALS  
Sequencing

1. Start 20', T 17-16	13 stop
2. St. 13', T 15-14	11 stop
3. St 16, T 12	8 stop
4. St 13, T 9	5 stop

saved reactions  
on ice.

poured sequencing  
gel

ALS 1-3A 1-4.

loaded sequencing gel - GATC

1' 2' 3' 20' 4'

run gel 1500 V, ~ 90 min

BpB 1 inch from bottom.

002230



12-13-96

Set up drying - 1PM left.

Returned 9:30 PM. Gel is dry.

Set up on film, RT O/N.

Kupp.

12-14-96

Developed seq. gel. 1-3 look great, #4 no bands.

Read gel

#1 ACCAATCTCGTCAgTggcctcgccgaccgctactgga  
tagcg tccccattgttgc.tat aacagg tcaagt gcc  
acgtaggat gatcgg tactgatgc tttcagg aaactcc  
gattgttgaggtaagct agatc gattacc aagca ...

#2 identical to #1

#3 ccaatctegtcagggcctcgccgacgcgctac  
tggatagcg tccccattgttgc.tataacagg tcaagc  
tgccacgtaggat gatcgg tactgatgc tttcagg  
aaactctgattgttgaggtaagct agatc gattacc aagca ...

#1 & 2 are SuRB (class 2)

#3 is SuRA (class I).

These PCR products are derived from an annealing  
temp of 58°C.

Xerox of gel, next page.

002231

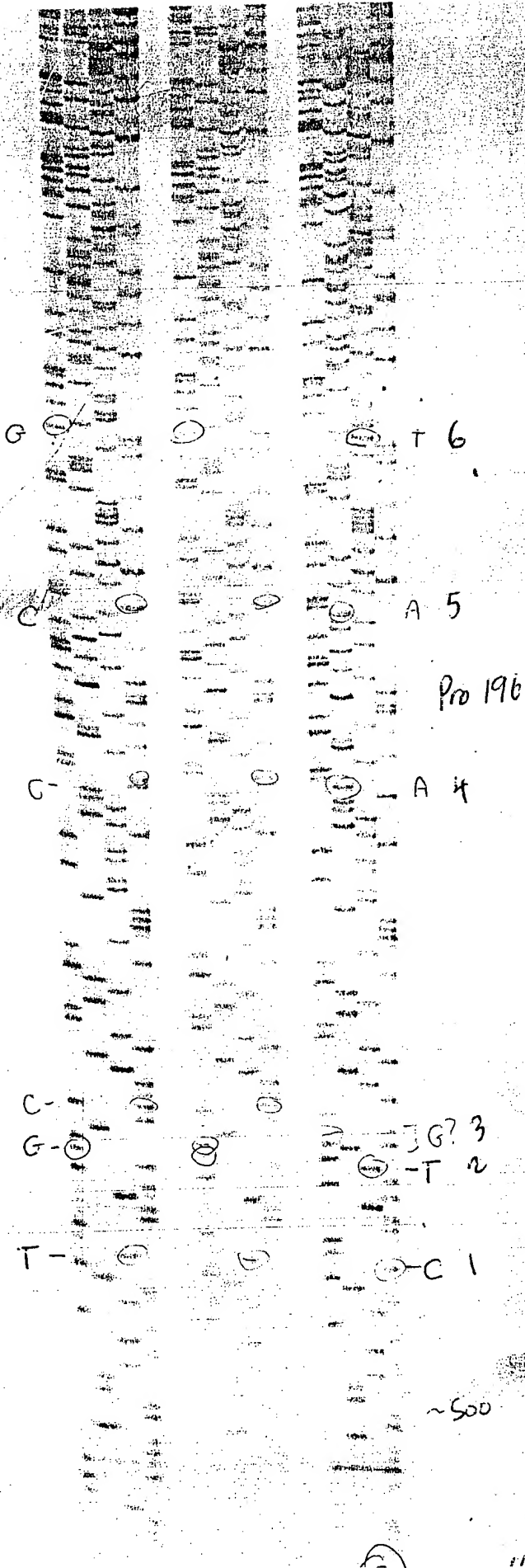
12-14-96

ALS-PGA sequence 1-4 O/N Exp 12-

12/14

Wrong primers I.

#3 in SuRA,  
the class I  
allele.



002232

Kupp.

~~12-16-96~~  
12-13-96

Kill curve data -

made NT-1 cell media

10 ppb (10 ng/mL) 1 ppm (1  $\mu$ g/mL)  
50 ppb (50 ng/mL) 5 ppm (5  $\mu$ g/mL)  
250 ppb (250 ng/mL) 10 ppm (10  $\mu$ g/mL)

~~made 100  $\mu$ g/mL gleam stock~~

~~add 1:100 dilution  $\Rightarrow$  10 ng/mL (10 ng/ $\mu$ L)~~

made 100 ng/mL gleam stock

and 1:100 1 ng/mL stock (1 ng/ $\mu$ L)

Began w/ 275 mL NT-1 media  
- added 2.75  $\mu$ L 1:100 dilution  
- mixed

- 4 no gleam plates

poured 4 10 ppb plates

- added 9  $\mu$ L 1:100 stock to bring gleam conc. to 50 ppb  $\rightarrow$  poured 4  
- added 40  $\mu$ L 1:100 stock " " " " 250 ppb  $\rightarrow$  poured 4

- added conc. stock to 1 ppm

- added conc. stock to 5 ppm

- added conc. stock to 10 ppm

each time taking  
the new volume into  
account.

allowed media dry time 4 hrs

plated 6 drops NT-1 cell liquid culture onto plates, 2 each

~~waited to spread spread~~ wrapped and placed  
~~air dried~~ in light room

Kipp

002233

12-16-96

The kill curve is contaminated. The cells have some apparent bacterial growth on them.

I set the kill curve up again~~st~~ w/ the leftover media:

6 drops NT-1 cells (NSH)  
swirled to spread  
air dried 1 hr in hood  
wrapped and placed in light room.

### ALS Genomic PCR -

As before - 30  $\mu$ L NT DNA ( $\phi$ -OH/ $\text{CHCl}_3$  after RNasing, vial'd)

two parallel reactions "C" & "D", using primers 1 & 3

100  $\mu$ L volumes

cycle cond: 92 1  
62 1 x 25  
72 1

increased annealing  
temperature 4°C to  
try to shift preference  
toward SurA locus.

reaction began at 9:40 AM.

The reactions finished at ~12 noon. I removed 20  $\mu$ L aliquots and loaded them into 0.5xTBE.

I purified the remaining 80  $\mu$ L (after  $\text{CHCl}_3$  xt) using the PCR prep kit.

- eluted DVAs in 40  $\mu$ L warmed (45°C)  $\text{dH}_2\text{O}$ .

I set up digests: 3  $\mu$ L PKs  
23  $\mu$ L ALS 1-3 C  $\rightarrow$  Kpm  
23  $\mu$ L ALS 1-3 D buffers, total volumes 30  $\mu$ L.

The digests began at ~1 PM.

Gel shows both reactions amplified at ~0.5 Kb band -  
no photo, will run further.

12-16-96

After 90 min digestion, 1  $\phi$ OH/CitCl<sub>3</sub> and ppt w/ NaOH + EtOH  
 - at -80°C 20'

Spin 15' RT, washed 70% EtOH, spin, speed vac'd pellet.

resusp 15  $\mu$ L dH<sub>2</sub>O, digested BmHI (buffer E) vol = 20  $\mu$ L

digested ~ 75 min, brought volume up to 100.

cleaned up digest by running through spin column

-  $\phi$ OH/CitCl<sub>3</sub> xt again

- ppt as before

- spin, washed

- resusp 20  $\mu$ L dH<sub>2</sub>O

set up ligations (7PM)

1 - 1  $\mu$ L PKs K/B

2 - 1-3C 1  $\mu$ L + 1  $\mu$ L PKs K/B

3 - 1-3D 1  $\mu$ L + 1  $\mu$ L PKs K/B

10  $\mu$ L total.

ligations at RT ~ 7:15 PM.

Elp'd 2  $\mu$ L each ligation at 10PM - and 1-3A  
 $t_c = 4.6$  for each.

after ~ 30 min recovery at 37°C, plated all cells on  
 LBA + X-gal / IPTG

- spread X-gal 10 min prior to plating (50  $\mu$ L)

- IPTG into cells (~ 10  $\mu$ L).

plates at 37°C O/N.

Kipp

12-17-96

All the NT-1 cells in the kill curve appear dry on the plates. It's hard to tell if they are growing.

12-17-96

at ~10AM there are colonies on the 1-3C and 1-3A plates, both blue and white.

- no colonies on 1-3D or PKS.

the 1-3C plate is very dense, > 750.

1 let the plates grow until ~ 1PM, then placed them at 4°C to allow the blue color to develop.

1 made 15 plates of NF-1 media.

- plated ~ 0.5ml thick NT (NSH) slurry on the plates to use in the shooting tomorrow with the ALS constructs.

1 inoculated 10 5ml LBA cultures

- 2 1-3 As

- 8 1-3 Cs → will sequence

1 ordered oligos from Genelink for the GFP strategy as discussed with Ramesh:

GFP Mutagenesis: create deletion  
engineer stop codon

GFP Δ:

5' ATCC ATG GTG AGC AAG GGC AG GAG C 3'  
NcoI frame shift.

GFP stop:

5' ATCC ATG GTG AGC AAG GGC TAG GAG C 3'  
NcoI STOP

GFP 3':

5' TTGAGCTCTTACTTGTACTGCTCGTCC 3'  
SacI

002236

12-17-76.

1 plated 10 NT-1 plates - very dense slurry. left ON in the hood to dry - to be shot tomorrow.

Kpp.

12-18-96

Plated Placed 10 minipreps (cultures) at  $4^{\circ}\text{C}$  for storage.

Prepared chimerics: Resuspended each chimera at 60ng/ml. (from 250ng/ $\mu$ l stock).

I do not know the identity of the chimeras, but they are labeled:

	DNA	-	resusp.			(OD)
1			528 $\mu$ L	dH <sub>2</sub> O		(4.0 OD)
			554 $\mu$ L			(4.2 OD)
2			772 $\mu$ L			(5.86 OD)
3			488.4 $\mu$ L			(3.7 OD)

12-18-96

Regardless of F, I proceeded w/ the shooting.

One Acc  
one F only

2 - 0

2 - 1

2 - 2

2 - 3

ten shots in all at Brokech.

After shooting moved cells to flashes. Added 10  $\mu$ l of NT-1 media (no selection) to cells, pipetted into flashes.

Wrapped plates to allow recovery of remaining cells.

JM. will plate cells onto glean media at 30 ppb or depending upon the outcome of the full curve.

The oligos arrived from Genetech.

Kyp

12-30-96 (return from break)

The plates are variable. JM plated them onto big plates on 12-20.

#1 A 3 B- dead cells, no apparent growth.

#2 A 3 B- 2A has very little media, and there are two fungal spores growing on it. However, separately. There are a few spots that may be regenerating.

B- There are a few spots on 2B also.



12-30-97

3A3B- no growth

DA3B- There is growth on both plates. There is sig. growth on plate A, which was plated at very high density.

AU - no growth

F - no growth

Because the densities are variable, it is difficult to determine if the cells are growing off of dead cell debris or if they are actually resistant to the gleam. I will need to move them to fresh plates.

Furthermore, I do not yet know the identities of the chimeras.

I will let the chimera treated cells grow longer before I transfer them.

Kipp.

1-2-97

GFP strategy - I have oligos to make mutations, and to amplify from the 3' end. I do not have a 5' oligo against the wild type, so I will order one and do all 3 PCRs in concert.

GFP 5'

5' GGATCC ATGGTGAGC AAGG 3' (19-mer)  
NcoI

$T_m \sim 55^\circ\text{C}$

from GeneLink.

002239

made NT-1 liquid media + 1- 30 ppb gleam.

1-3-97

Passaged NT-1 cells into liquid culture.

Kupp.

1-6-97

GFP 5' oligo arrived from GeneLink:

Lane	Oligo ID	$T_m$	Size	Total A <sub>260</sub> Units Supplied
1	GFP 5' delta	65.5	25	8.21
2	GFP 5' stop	65.5	26	8.32
3	GFP 3' Sac	62.4	27	8.04
4	GFP 5' NcoI	51	19	6.82
5				
6				
7				
8				
9				
10				

Mobility of an oligonucleotide is dependent upon the size and

$$1: \frac{8.21 \times 90}{25} = 29.6 \text{ nmol}$$

$$2: \frac{8.32 \times 90}{26} = 28.8 \text{ nmol}$$

$$3: \frac{8.04 \times 90}{27} = 26.8 \text{ nmol}$$

$$4: \frac{6.82 \times 90}{19} = 32.3 \text{ nmol}$$

03:31:4

Yields a bit low, but clearly sufficient.

Resusp each oligo in 10x nmol vol. of dH<sub>2</sub>O  
(ie #1 Resusp in 296 µl dH<sub>2</sub>O → 100 µM).

I used GFP-KS as the substrate for the PCR:

a 1:500 dilution

GFP PCR for chimeras: 50 µl rxns.

3 reactions, in duplicate

used 3 µl 1:500 plasmid DNA.

002240

1-6-97

Made premix, enough for 8 rxns.

10x	5 $\mu$ L	40
MgCl <sub>2</sub> (25mM)	3 "	24
dNTPs (1.25mM)	8 "	64
oligo 1 (GFP Sac 3')	0.5 " x 8	4
tag (~100bp)	0.5 "	4
dH <sub>2</sub> O	29.5 "	236
	<u>46.5 <math>\mu</math>L</u>	

oligo 2 added separately to each rxn.

Added 46.5  $\mu$ L premix to each PCR rxn, then added 0.5  $\mu$ L of the second oligo.

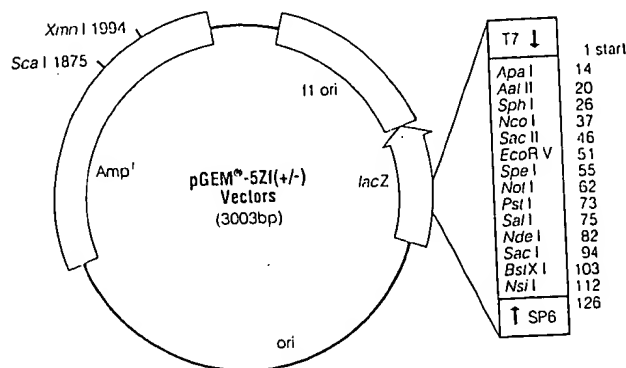
GFP → 2 tubes  
GFP  $\Delta$  →  
GFP stop →

92°C 1  
48°C 1 x 25  
72°C 1

The Tms shown on p138 are for the full length oligo - initially a portion (the RE site) is not homologous.

The PCR began at 5 PM, linked to 4°C soak.

I inoculated a 5ml culture of pGEM5ZF. This plasmid (LR) has both NcoI and SacI sites in its polylinker and will allow straightforward cloning of the GFP PCR products.



Promega.

Amp<sup>R</sup>

B/W selection.

002241

Epp

1-7-97

Plasmid prep'd pGEM 52f 2x 2mL (made frozen stock).

Q-DH / CHCl<sub>3</sub> xt.

ppt. separately in EtOH.

Resusp. pellets in 50  $\mu$ L each, combined.

Digested 4  $\mu$ L pGEM  
3  $\mu$ L (3  $\mu$ g) iBT 210.1

→ Sac I (buffer J) total volume 30  $\mu$ L  
1  $\mu$ L enzyme each.

at 37°C.

After 75 min heated samples to denature enzyme

- 65°C 15 min

- Q-DH / CHCl<sub>3</sub> (brought vol. to 100).

- ppt. w/ EtOH + NaOAc (1/3 vol 3M) at -80°C

Started gel of GFP PCR rxns -

loaded 5  $\mu$ L each rxn.

- Q-DH / CHCl<sub>3</sub> xt.

GFP	GFP	GFP $\Delta$	GFP $\Delta$	GFP stop	GFP stop	U	Std.
1	2	1	2	1	2		

After loading the gel w/ 5  $\mu$ L, I Wizard prep  
cleaned the remaining 45  $\mu$ L of each #1  
I eluted these DNAs in 40  $\mu$ L warm dH<sub>2</sub>O.

I began SacI digests of the cleaned PCRs - ~~prior to~~  
knowing w/ certainty that the rxn worked.

GFP #1

GFP  $\Delta$  #1

GFP stop #2

→ I checked the gel after ~20 min

7.5  $\mu$ L DNA each. total volume = 30  $\mu$ L

002242

1-7-97

I did not purify the PCR products from the rxns #2, other than  $\text{CHCl}_3$  xt.

The PCR frag. digests were set up ~25 min after the vector digests.

- after 75 min the digests were heated to  $65^\circ\text{C}$  and ppt. in EtOH + 3M NaOAc. at  $-80^\circ\text{C}$ .

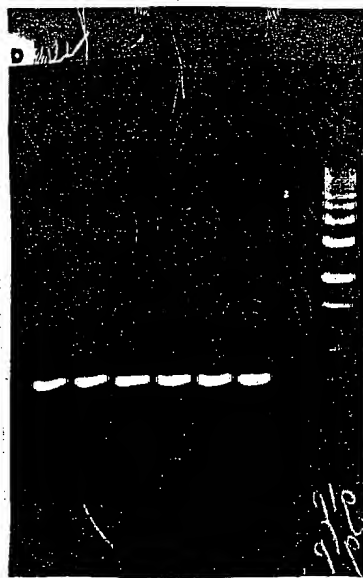
Spin, washed and dried vectors.

Resusp.<sup>each</sup> in  $25\ \mu\text{L}$   $\text{dH}_2\text{O}$ . Digested w/  $1\ \mu\text{L}$  NcoI in buffer D.  
- total vol = 30.

Spin, washed and dried PCR frags.

Resusp.<sup>each</sup> in  $26\ \mu\text{L}$   $\text{dH}_2\text{O}$ . Digested w/  $1\ \mu\text{L}$  NcoI in buffer D.  
total vol = 30  $\mu\text{L}$ .

Mutagenic PCR  
to create GFP $\Delta$ ,  
GFP stop.



lanes 1+2 : GFP

lanes 3+4 : GFP  $\Delta$

lanes 5+6 : GFP stop.

After 75 min digestion time I heated the NcoI digests to  $65^\circ\text{C}$  for 15 min to denature the enzyme.

I ppt. all the frags. in EtOH by  $\uparrow$  the vol. to  $100\ \mu\text{L}$  w/  $\text{dH}_2\text{O}$  then adding  $33\ \mu\text{L}$  3M NaOAc +  $300\ \mu\text{L}$  100% EtOH.

- vtx briefly
- ppt at  $-80^\circ\text{C}$

002243

1-7-98

### ALS Regenerants from 12-18 shooting-

I moved various apparently viable calli to NT-1 solid media + 30 ppb (30  $\mu\text{g/L}$ ) gleam.

(1-6) ALS 2-A 11 calli

1-7 ALS D A 9 calli

ALS D B 10

ALS 2-B 9

These calli look similar to regenerants from an Agrob. TF growing on Kan selection. They are small raised friable looking tissue, typically a yellowish clear color.

Kipp

1-8-97

### GFP-PCR cloning-

I spun, washed and dried the vectors and fragments.

I resusp. the vectors in 20  $\mu\text{L}$  dH<sub>2</sub>O and the frags. in 10  $\mu\text{L}$ .

I set up <sup>7</sup> ligations:

gfp 210	}	1.5 $\mu\text{L}$ insert, 1 $\mu\text{L}$ vector
gfp gem		
gfp 210 $\Delta$		
gfp gem $\Delta$		
gfp 210 stop		
gfp gem stop		

210.1 vector alone - 1  $\mu\text{L}$  vector

Each ligation has a 10  $\mu\text{L}$  total volume, and used ~0.75  $\mu\text{L}$  ligase

Ligations proceed ~~at~~ at RT, beginning at 3 PM.

002244

1-8-97

TF DH5 $\alpha$  via Elp'n at 9PM -

spread X-gal + IPTG on 4 plates (of 2)

$t_c = 4.5$  for all the tfs.

plated all the cells after 40 min recovery at 37°C.

2105 on X-gal + LBA.

pGENs on LBA

at 37°C O/N.

Kipp.

1-9-97

developed

At 10 AM, there are no colonies yet, but I can see some bumps forming.

Also I realized that I plated the pGENs on LBA w/o X-gal so the Blue selection isn't going to work.

I allowed the plates to continue incubating.

ALS Chimera - I decided to plate some regenerating tissue from the Au control plate on NT + 30 ppb to demonstrate the selective process.

I selected 8 calli from the original plate. Each of these were growing in an area of high cell density, and therefore could be escapes -

In addition I plated HB50 B7 - a Kan resistant stably transformed cell line on 30 ppb.

The other plates seem to be showing some resilience to 30 ppb glean. The calli look about the same - I can't tell if they've grown since Tuesday.

002245

1-9-97

### ALS PCR Optimization-

i have 10 clones that i want to sequence A5, A6, C1-C8.

A5 and A6 are derived from PCR at 58°C

C1-C8 are from PCR at 62°C

<sup>re-</sup>  
I inoculated 4 mL LBA cultures to grow fresh cells to plasmid prep and sequence.

### GFP-PCR mutagenesis clones-

The plates grew up well. There are many colonies on the BT210.1 vector alone plate.

∴ i will conduct a <sup>32</sup>P screen rather than a PCR screen.

i lifted the pGEM GFP Δ plate (Mauritis 1:100) lysed and fixed the cells stratalinked.

i saved the other plates to lift tomorrow, wrapped them and placed them at 4°C.

Kpp.

1-10-97

### GFP-PCR mutants-

i lifted the remaining 5 plates:

GFP 210

GFP gen

GFP 210 stop

gen stop

GFP 210 Δ

stratalinked

002246

i made a random-primed probe using 1.5 μL of the PCR rxn. from the GFP Sac-Nco PCR rxn.



1-10-97

In making this probe I considered that a small amount of the template DNA may be present (GFP KS, pKS backbone) which could lead to higher background.

I placed the 6 filters in the same small Hybe bottle with ~20 mL Hybe buffer.

I denatured the 50  $\mu$ L probe after removing dNTPs  
- probe > 5 on 100x setting.

used all of the probe.

Hybe began ~12:30 PM.

The following arrived from Kimeragen:

I) sickle cell plasmids (in pT7 blue, Novagen)

$\beta$ s - sickle phenotype

$\beta$ N - normal

BA has Bsu 36 I site that is not present in  $\beta$ s

We anticipate using these plasmids to attempt in vitro mutagenesis using NT-1 UV treated plant extracts.

II) Primers: BG-03 TCC TAA GCC AGT GCC AGA AGA

BG-05 CTA TTG GTC TCC TTA AAC CTG

It is unclear where these primers bind, but I suspect that they flank the coding region.

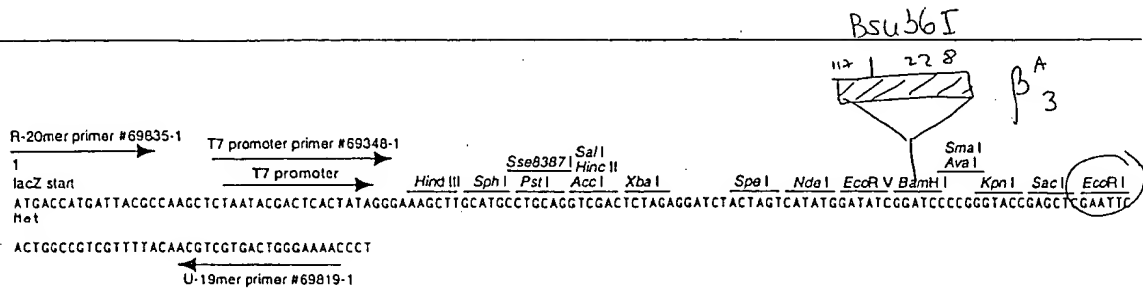
A photocopy of the sheet sent follows on the next page.

We did not receive the chimeras at this time.

They will be sent later.

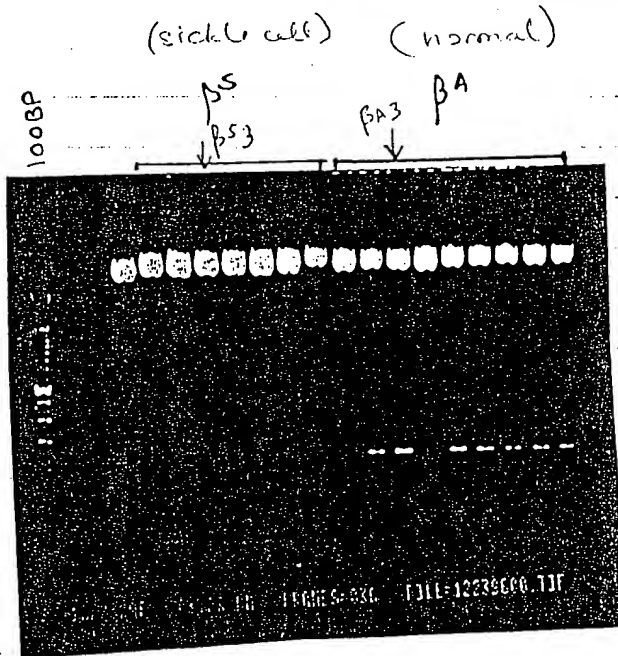
1-10-97

$\beta_3^S$  and  $\beta_3^A$  cloned into pT7(Novagen) (25 plaques)



pT7Blue cloning/expression region

Novagen • ORDERING 800-526-7319 • TECHNICAL SUPPORT 800-207-0144



mini preps cut w/ Bsu36I and EcoRI

← 253 bp.

PRIMERS: 1.3 µg/µl (10 µl ea)  
BG02: TCC TAA GCC AG  
GCC AGA AGA  
BG05: CTA TTG GTC TCC  
TTA AAC (TG-

Conversation w/ Naomi Thompson (K- 215 504 4444 x 111)

Sequencing plasmid: 500 ng - 1 µg dried

5 pmol primer / rxn.

002248

1-10-97

for PCR products - gel purify if not single product

or if single band use chromatography to purify

supply 10ng / 100 bases to be sequenced

will email results - considers Sequencher Software package

→ Howard Cash, (313) 769 7249

ALS plasmid preps - PCR C (62)

I prepped the 10 plasmids A5, A6, C1-C8 using the Promega Wizard prep kit.

- eluted plasmids in 50  $\mu$ l dH<sub>2</sub>O.

- turned UV lamp on.

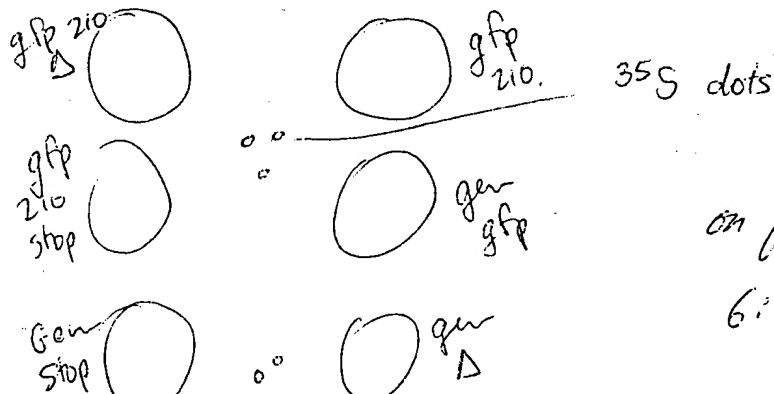
GFP-PCR hybe - began washing filters at 5PM (4½ hrs hybe).

2x in wash 1 (20-25 min each)

2x in wash 2 (20-25 min each)

air dried filters 5 min, affixed to Immobilon paper

I realized that I neglected to punch alignment holes in the GFP 210 stop membrane, so it will be very tough to line it up with the plate.



002249

1-10-97

UV readings on ALS-PCR plasmids to be sequenced:

7.0000		2 $\mu$ L = 100		
SAMPLE	A280	A260	280/260	260/280
1.0000	0.0383	0.0716	0.5352	1.8685 C1
2.0000	0.0239	0.0434	0.5512	1.8144 C2
3.0000	0.0103	0.0190	0.5432	1.8411 C3
4.0000	0.0395	0.0721	0.5477	1.8257 C4
5.0000	0.0358	0.0623	0.5751	1.7388 C5
6.0000	0.0370	0.0630	0.5873	1.7027 C6
7.0000	0.0414	0.0715	0.5789	1.7275 C7
8.0000	0.0537	0.0964	0.5568	1.7958 C8
9.0000	0.0494	0.0871	0.5548	1.8026 A5
10.0000	0.0233	0.0406	0.5744	1.7410 A6

ALS PCR plasmid  
Subclones

actual [ ]

2.5x A260

in  $\mu$ g/ $\mu$ L.

The yields of C2, C3 and A6 are low. I re-inoculated LB+Amp cultures (5mL) to grow again overnight, also pA and pS (sickle cell cultures).

Kipp

1-11-97

I developed the film - facing page.

GFP-PCR screen: selected putative clones to PCR screen.

210 gfp 6 + 3 controls 3 controls: 210.1, GFP-KS, pgen

pgen gfp 6

210 stop 3

pgen stop 4

33 rxns in all  $\rightarrow$  38

210  $\Delta$  4

gen  $\Delta$  7

1.5  $MgCl_2$  (25mM)

4 dNTPs

2.5 10x

0.2 prim. 1 3'Sac

0.2 tag

10.3 dH<sub>2</sub>O

18.8

57 gfp  $\frac{15}{33} = .455 \rightarrow 325.1$  PM gfp

152

95

7.6

7.6

391.4

714.4

stop  $\frac{1}{33} = .212 \rightarrow 151.5$  PM stop

$\Delta$   $\frac{11}{33} = .33 \rightarrow 236.1$  PM  $\Delta$

002250

added 3  $\mu$ L Nco 5' GFP to PM gfp

added 1.4  $\mu$ L Nco 5' stop to PM stop

added 2.2  $\mu$ L Nco 5'  $\Delta$  to PM  $\Delta$

92 1

45 1 x 25

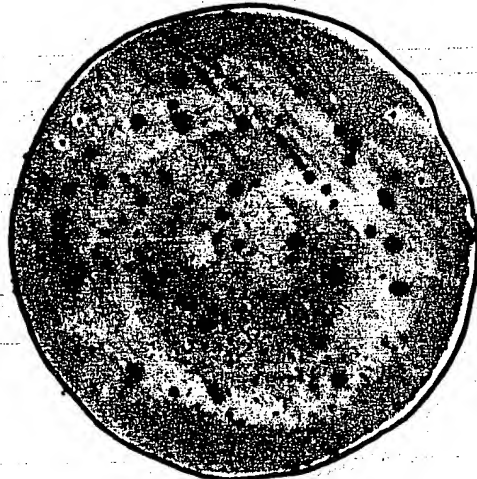
72 1

link to 4°C.

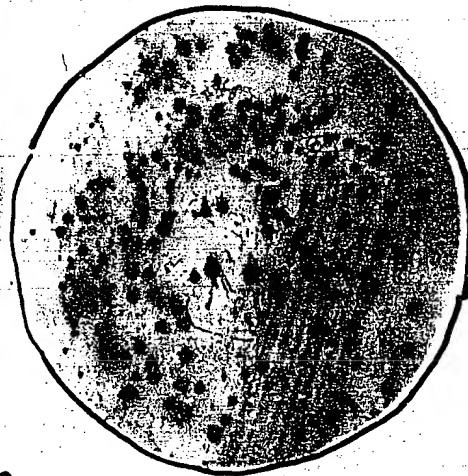
1-11-97

GFP-PCR Subclones Colony Hybe  
1-11-97  
20 hrs Rt.

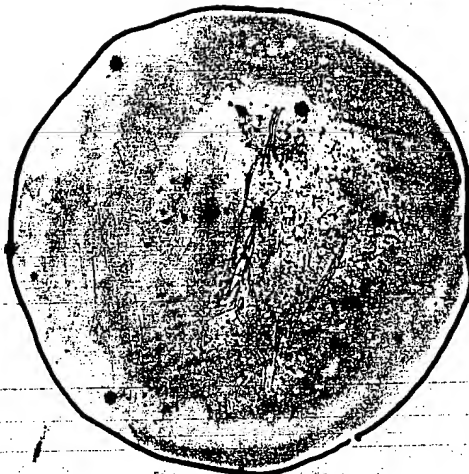
gfp  
210  
Δ



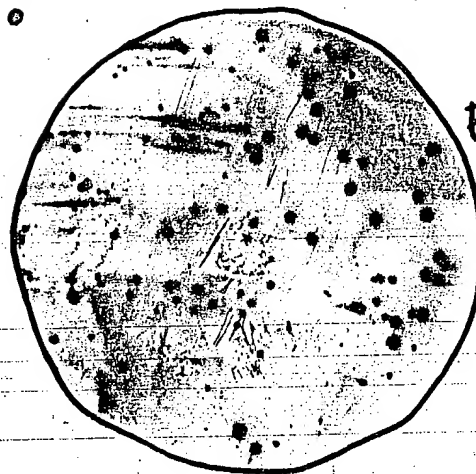
gfp  
210



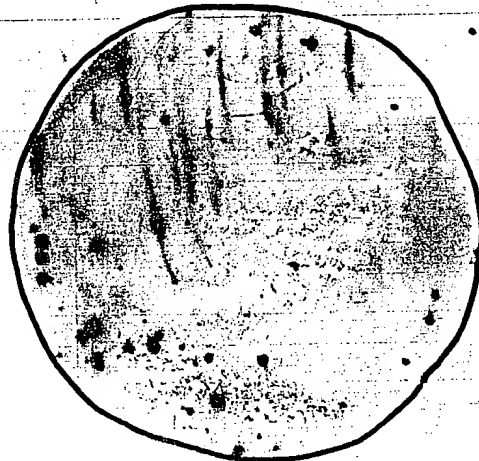
gfp  
210  
stop



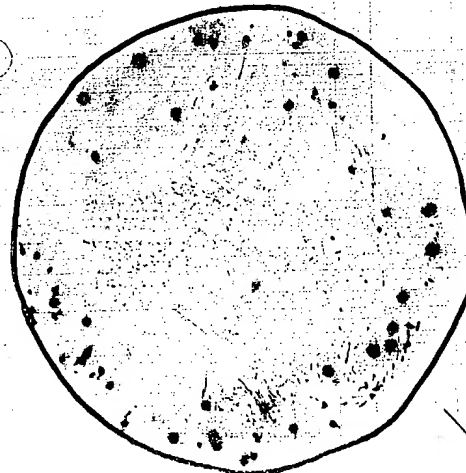
p<sub>gem</sub>  
GFP



p<sub>gem</sub>  
GFP  
stop



p<sub>gem</sub>  
GFP  
Δ



002251

→ Saved leftover cells at 4°C. Will run samples tomorrow.  
moved cultures from 1-10 to 4°C.

16.

1-12-97

loaded PCR gel of GFP subclones.

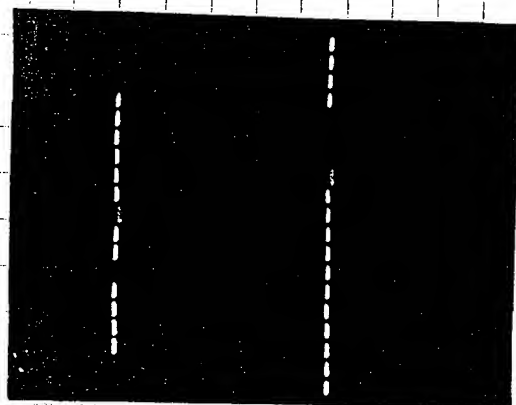
top: 210.1 pGEM GFP-KS GFP210 1-6 Std gem 1-6

bottom: 210  $\Delta$  1-4 gem  $\Delta$  1-7 Std. 210 stop 1-3 gem stop 1-4

(noticed that GFP210  $\Delta$  4 has no mineral oil on it.

PCR Screen  
of GFP-mutants

ID +s for:  
pGEM GFP  
p 210 GFP  
p GEM GFP  $\Delta$   
p 210 GFP  $\Delta$   
p GEM GFP stop



top

bottom

Plasmid preped 3 mL of C2, C3 and A6 ALS subclones.  
- via kit, resusp 50  $\mu$ L dH<sub>2</sub>O

SAMPLE	A280	A260	280/260	260/280	
1.0000	0.0719	0.1267	0.5677	1.7614	<del>A6</del>
2.0000	0.0770	0.1464	0.5400	1.8517	<del>C2</del>
3.0000	0.0650	0.1167	0.5565	1.7968	<del>A6</del> C3

2  $\mu$ L  $\rightarrow$  100

Much better yields. Marked in green to distinguish  
from those preped the other day.

I inoculated the following cultures:

210 1	210 $\Delta$ 1	210 stop 1	} $\rightarrow$ just a guess, I tried to align the filter based upon background signal.
210 2	210 $\Delta$ 2	210 stop 2	
210 3	210 $\Delta$ 3		
gem 1	gem $\Delta$ 1	gem stop 1	
gem 2	gem $\Delta$ 2	gem stop 2	
gem 3	gem $\Delta$ 3	gem stop 3	

002252

Kipp.

1-13-97

All the cultures grew well. I performed PCR on the 2 GFP 210 steps using the standard PCR colony screen protocol. I did not include a control.

ALS Sequencing by C-tracking - distinguish SARA/RB via 4 Cs  
see seq. pg 113

12 samples, ALS 1-3 A 2 (B) A5, A6, C1-C8.  
ALS 1-3 A 3 (A)

~~to correct~~ The rxn. requires  $\frac{1}{4}$  vol. of the components.  
I used 7.5  $\mu$ L DNA for A2, A3, C2, C3, A6 } roughly 2  $\mu$ g.  
10  $\mu$ L DNA for C1, C4-C8

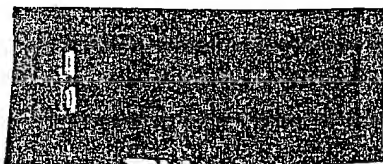
denatured each in 20  $\mu$ L total volume using 2mM EDTA / 2m NaOH.  
- open, washed and resusp. each in 25  $\mu$ L dH<sub>2</sub>O.  
- added 0.25  $\mu$ L ALS 1 (1  $\mu$ M).  
- annealed 30 min at 37°C.

Made premix w/ enz, enough for 14 rxns. / 4  
- add  $\frac{1}{4}$  regular amount, so I made enough PM for 4 full rxns.  
2  $\mu$ L 1410 labelling mix  
1  $\mu$ L DTT + 4-  
0.5  $\mu$ L d<sup>35</sup>S  
2  $\mu$ L 1:8 Sequenase  
1.4  $\mu$ L

Added <sup>a</sup>mix to each rxn, then added 0.5  $\mu$ L d<sup>35</sup>CTP to each.  
- added stop buffer (2  $\mu$ L) after ~ 4 min.

loaded into seq. gel: A, B, A5 A6, C1-C8  
- ran gel at ~1500 V, 1:20 min  
- ff, dried and set up on film

PCR screen  
of GFP



GFP 210 stop 2 → negative.  
GFP 210 stop 1

Kipp.

002253

1-14-97

C. trachomatis isolated not  
attached

NAME P. Kipp	EXP. NO.	ASSAY
PURPOSE plasmid prep GFP PCR transfer ALS putatives to 50 ppb rescreen 210 GFP	NOTES	
		DATE 1-14-97

Continued from "Intron-Mediated", Book 2, p 151.

~1.9 mL of  
I plasmid prep'd the 15 gfp PCR subclones and the pA and pS sickle cell plasmids via the Promega kit. (new)  
- 19 pps in all (12 ea. of pA/pS)  
- eluted DNAs in 50  $\mu$ L dH<sub>2</sub>O each.

GFP-PCR - I lifted the 210 GFP Stop plate to Nytran (SBS)  
- made alignment holes w/ 18 gauge needle.  
and conducted the colony lysis according to Mannatis (1:100).

8/30 attached DNA to membrane

During the lift, I prepared a GFP probe as before:

- 1.5  $\mu$ L GFP-PCR (Nco 5' - 3' Sac primer set) in 21  $\mu$ L dH<sub>2</sub>O
  - 20  $\mu$ L 25x Random primed buffer
  - 1  $\mu$ L dATP, dTTP, dGTP
  - 5  $\mu$ L 32P-dCTP
  - 1  $\mu$ L klenow ex-3
- } B-1 kit

I heated the DNA to 95°C for 3 min before starting the reaction -

Incubated 30 min at 37°C

Began prehybe with membrane at ~12noon.

Removed unincorporated dNTPs from probe synthesis using G-50 size exclusion column.

- probe very hot

002254

Heated probe to ~95°C, 3 min. Used 15  $\mu$ L (of 50)  
to probe blot - Began 12:25 PM.

SIGNATURE

P. Kipp



stored remainder of probe at  $-20^{\circ}\text{C}$

### ALS-Chimera Shooting Expt.

- I transferred some of the regenerating material to 50 ppb
- I selected 8 new cells from the ALS-DB plate and 4 An, and moved them to a 12 well plate with  $\text{NR} = 50 \text{ ppb}$  glean.
  - selection of +/- chimera simultaneously

GFP-PCR - I began washing the GFP 210 stop colony hybridization at  $-5 \text{ PM}$ .

- 2x 1m wash 1 (20-25 min each,  $\sim 30 \text{ mL}$ )
- 2x 1m wash 2 ( " " )

The membrane has counts. I taped it to 1mm paper wrapped it in Aera wrap, and set it up on film, in the  $-80^{\circ}\text{C}$ .

Kipp

002255

SIGNATURE

Sequence ALS C1-C8  
dev GFP 210 stop, inoculate

1-15-97

### ALS genomic PCR cloned products -

I will sequence the 8 subclones from the "C" PCR reaction (62°C)

I need ~5mg each plasmid (A260s, pg 148, 150 book 1M #2)

- I used 25  $\mu$ L C1, C4, C5, C6, C7, C8 (p148)

- I used 15  $\mu$ L C2, C3 (p150)

I set up the denaturation reaction in 30  $\mu$ L volumes.

- 3  $\mu$ L 2mM EDTA / 2M NaOH

+ DNA

+ dH<sub>2</sub>O  $\rightarrow$  30

30' at 37°C.

ppt, brought vol to 100  $\mu$ L, added 3M NaOAc + 300  $\mu$ L 100% EtOH.  
- at -80°C.

Spin after 25' at -80°C, 15' at 14K.

- washed 70% EtOH

- speed vac'd  $\rightarrow$  resusp. each DNA in 7  $\mu$ L dH<sub>2</sub>O

Added 2  $\mu$ L 5 $\times$  reaction buffer and 1  $\mu$ L (1  $\mu$ mol) ALS1  
- 30' at 37°C to anneal

Followed Sequenase protocol  
to terminate

During annealing, I poured  
a 6% sequencing gel

Once the rxns. were stopped,  
I placed them on ice.

15 min pre-run

20 begin 1 17 term 12 stop

18 begin 2 15 term 10 stop

16 begin 3 13 term 8 stop

14 begin 4 11 term 7 stop

9 begin 5 5 term 0 stop

5 begin 6 2 term -3 stop

3 begin 7 0 term -5 stop

1 begin 8 -2 term -7 stop

SIGNATURE

Peter Kipp

002256

PURPOSE

Sequence ALS genomic clones  
GFP210 Stop dev.

NOTES

cont 1-15-97

DATE

1-15-97

I loaded the sequencing gel "GATC", with C1 at the left.  
 - after C1-C5 were loaded, I started the gel for 5 min  
 - I stopped the gel, shipped one lane, then loaded C6-C8

Each sample was heated to 90°C for 3 min prior to loading.  
 - the gel ran at ~1500 V.

GFP-PCR - I developed the colony lift -

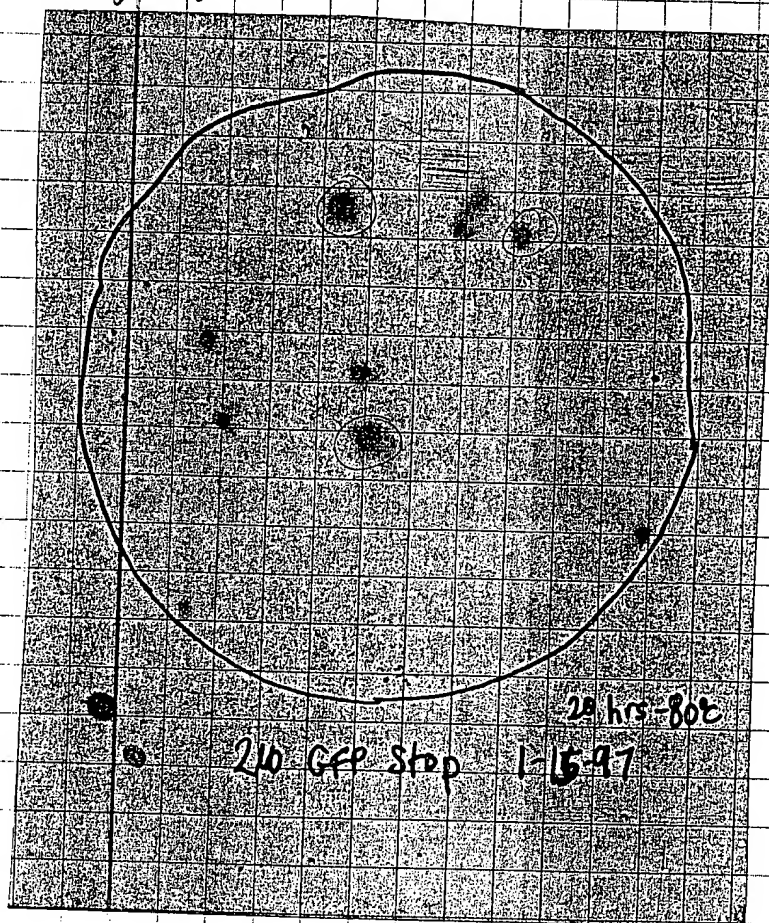
I inoculated 3 of the colonies that lined up in YENB + Amp.

ALS Chimera Expts -

I inoculated two flasks of fresh NT-1 cells to make protoplasts Friday.

I gave them a heavy inoculum, 5 mL → 45 mL

I checked the growth of the shot materials. The large plates are fully overgrown. The calli that I moved to 30 ppb media are starting to look a bit different. The ALS 2A plate #3 looks like it is growing well. The callus is translucent and looks wet and healthy. Also ALS DB #6 and #7 look quite healthy, although have't grown as much as 2-3.



24 hrs - 80°C

240 GFP Stop 1-15-97

002257

SIGNATURE

PURPOSE

Sag ALS genomic PCR's (62C)  
GFP 2nd stop

NOTES

Cont 1-15-97

DATE

1-15-97

I stopped the sequencing gel when the Bpb dye was ~ 2mm from the bottom.

- transferred gel to 3MM Whatman
- wrapped in saran wrap
- dried at 80°C ~ 1hr under vacuum.
- set up on film at RT
- geyser registered some counts

Begin 1-16-97

Kupp.

I developed the sequencing gel for ALS 1-3C 1-8. There are no bands - the sequencing failed. Perhaps the reagents in the kit were too old. (film not attached).

plasmid prep

This morning GM transferred the regenerating NT-1 materials from the shooting to 50 ppb in 12 well plates. He also selected a few calli from the initial large plates, after which the large plates were discarded.

Met w/ Greg to discuss projects: outline

DNA: protein - binding profile of chimera / all DNA w/  $\Delta U^{+/-}$  extracts

- radiolabel chimeras w/  $\gamma$ -ATP
- strand transfer assay

ALS - Electroporation of chimeras into NT-1 protoplasts

- Rhodamine-labelled chimeras included
- genomic PCR of samples in regeneration (Sun/Mon.)

GFP - Send  $\Delta$  stop and wt for sequencing

- create Agro vectors and +F NT-1

002258

SIGNATURE

Rhodamine label ALS chimeras  
Organizational meeting w/ Greg

cont 1-16-97

1-16-97

Meeting w/ Greg cont:Sickle Cell: plasmids ready, no chimeras available yetIsolation of Nuclear Extracts from NT-1 -

We will isolate UV +/- nuclear extracts from NT-1 cells next wednesday (1-22). I will inoculate ~ 400 ml of NT-1 into liquid culture on Saturday (1-18). Ideally by wednesday we would like to have approximately 100ml packed volume of cells in active cell division.

Fluorescent Labeling of ALS-2 and ALS-D:

protocol: (from Kimerager) see blue 3 ring binder.

briefly: Use terminal transferase to add fluorescently tagged nucleotide analogs to the 3' end of molecules.

to label 100 pmoles chimera:

4  $\mu$ L TdT 5x rxn. buffer4  $\mu$ L 25mM  $\text{COCl}_2$ 1  $\mu$ L 1mM Fluorescein-12-dUTP (1mM  $(\text{CH}_3)_4$  rhodamine-6-2'-dUTP) $\mu$ L chimera $\mu$ L TdT (50U) $\mu$ L  $\text{dH}_2\text{O}$ 20  $\mu$ L

BMB cat # 1534 378

mix components, pulse spin  
incubate 15' at 37°C, transfer to ice

add: 2  $\mu$ L 0.2M EDTA pH 82.5  $\mu$ L 4M LiCl75  $\mu$ L 100% EtOH (-20°C)

ppt O/N, spin, wash 70%, air dry

002259

SIGNATURE

For research purposes only. Not for use in diagnostic procedures for clinical purposes. FOR IN VITRO USE ONLY.

# Tetramethylrhodamine-6-2'-deoxy-uridine-5'-triphosphate

(Tetramethylrhodamine-6-dUTP)

Tetramethylrhodamine-5(6)-amino-thiono-[5-(3-aminoallyl)-2'-deoxy-uridine-5'-triphosphate]

tetralithium salt

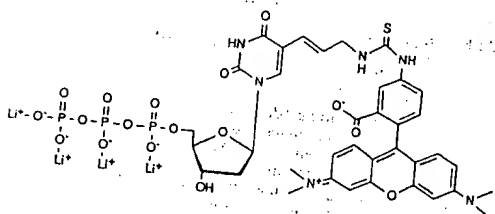
Cat. No. 1534 378

25 nmol (25 µl)

## Product description

Commercial availability: 1 mM solution.

Structural formula:



Formula:  $C_{30}H_{36}N_6O_{12}P_3Li_4$

Molecular weight: 959.4

The improved Tetramethylrhodamine-6-dUTP contains a tetramethylrhodamine derivative as fluorogenic moiety with superior properties. The use of the improved nonradioactive nucleotide for labeling of *in situ* probes will result in brighter, sharper and background-free *in situ* signals.

Spectral properties: Excitation<sub>max</sub>: 551 nm, emission<sub>max</sub>: 575 nm (0.1 M sodium borate buffer, pH 8.5)

Stability: store at -20°C, protected from light; a decomposition of approx. 5% may occur within 6 months.

## Application

Tetramethylrhodamine-6-dUTP is used for nonradioactive labeling of DNA. The modified nucleotide is a substrate for E. coli DNA polymerase I (holoenzyme and Klenow fragment), T4 and T7 DNA polymerase, Taq DNA polymerase and reverse transcriptase (e.g. from AMV and M-MuLV). It can replace dTTP in the nick-translation reaction as well as in the random primed labeling method for DNA labeling. The nucleotide also serves as a substrate for terminal transferase for DNA 3'-end labeling (1). Tetramethylrhodamine-labeled probes show red fluorescence. They are suited for use in *in situ* hybridization for direct fluorescence detection. Multiple fluorescence labeling using fluorescein-12-dUTP\* (yellow fluorescence) and aminomethylcoumarin-6-dUTP\* (bright-blue fluorescence) is possible.

## Standard labeling assay

DNA is labeled by random primed incorporation of tetramethylrhodamine-labeled deoxy-uridine-triphosphate with Klenow enzyme (2):

1. Required reagents
1. Control DNA, pBR 328\*, 200 µg/ml, linearized with Bam HI,
2. Hexanucleotide mixture\*, 10 x conc.,
3. dNTP\* labeling mixture, 10 x conc., containing:  
1 mM dATP, 1 mM dGTP, 1 mM dCTP, 0.65 mM dTTP, 0.35 mM tetramethylrhodamine-dUTP, pH 7.5 (20°C),
4. Klenow enzyme\*, labeling grade, 2 units/µl,
5. 0.2 M EDTA, pH 8.0 (20°C),
6. 4 M LiCl,
7. 70% Ethanol (v/v) and 100% (v/v),
8. TE buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 (20°C).

## II. Labeling reaction

10 ng to 3 µg linearized DNA can be labeled per standard reaction. Larger amounts may be labeled by increasing the reaction volume and components proportionally.

## Control and standard assay

1. It is recommended to purify the linearized DNA by phenol/chloroform extraction and ethanol precipitation.
2. Denature the DNA by heating in a boiling water bath for 10 min and quickly chilling on ice/NaCl. Complete denaturation is essential for efficient labeling.
3. Add the following to a microfuge tube on ice:  
1 µg of freshly denatured DNA or 5 µl control DNA, respectively  
2 µl hexanucleotide mixture  
2 µl dNTP labeling mixture  
fill up to 19 µl with sterile redist. water (control reaction requires 10 µl sterile redist. water) and add 1 µl Klenow enzyme.
4. Centrifuge briefly and incubate for at least 60 min at 37°C. Longer incubation (up to 20 h) can increase the amount of labeled DNA.
5. Stop the reaction by adding 2 µl EDTA solution.
6. Precipitate the labeled DNA with 2.5 µl LiCl and 75 µl prechilled (20°C) absolute ethanol. Mix well.
7. Leave for at least 30 min at -70°C or 2 h at -20°C.
8. Centrifuge (at 12 000 x g), wash the pellet with cold 70% ethanol, dry under vacuum and dissolve in 50 µl TE buffer.
9. Apply labeled DNA into *in situ* hybridization reaction.
10. The amount of newly synthesized labeled DNA depends on the amount and purity of the template DNA. In the standard reaction with 1 µg DNA per assay approx. 10% of the nucleotides are incorporated into about 250 ng of newly synthesized labeled DNA within 1 h and approx. 30% of the nucleotides into about 750 ng after 20 h.
11. If desired, the kinetics of the labeling reaction can be followed and the amount of newly synthesized labeled DNA determined by the addition of radioactively labeled tracer dNTP and trichloroacetic acid or ethanol precipitation.

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## References

- 1 Raap, A. K. et al. (1991) Exp. Cell Res., 194, 310
- 2 Feinberg, A. P. & Vogelstein, B. (1983) Anal. Biochem., 132, 6.

available from Boehringer Mannheim GmbH



BOEHRINGER  
MANNHEIM



002260



PURPOSE Rhodamine labeling of ALS2/0

NOTES cont 1-16-97 (p15)

DATE

1-16-97

Rhodamine labeling of ALS-2/0:(CH<sub>3</sub>)<sub>4</sub> Rhodamine-6-2'-dUTP (BMB sheet opposite page)Promega enzyme: (BTI freezer)

The promega enzyme is at 20 U/μl and its 5x rxn buffer already contains CoCl<sub>2</sub>. This will require modifying the protocol.

I will label 200 pmoles of ALS2 and ALS D - this way we have one that is all DNA and one hybrid.

Assuming that each chimera is 100 bases:

$$100 \text{ bases} \times \frac{330 \text{ g/mol}}{1 \text{ base}} = 33,000 \text{ g/mol} \quad 33,000 \text{ ng/mol} \times \frac{1 \text{ nmol}}{1000 \text{ pmol}} = 33 \text{ ng/pmol}$$

∴ 100 pmoles ≈ 3.3 μg of the chimera

I will use ~6 μg of each chimera for the reactions (~200 pmol/rxn.)  
- chimeras @ 250 ng/μl, ∴ use 25 μl each

I will conduct 2 x 200 pmol for each chimera, giving a total of ~12 μg of labeled chimera for each. For one electroporation/shooting I will use ~3 μg, so I am making enough to do 4 electroporations or shootings.

Reaction con'd:

	8 μl	5x TdT buffer
	2 μl	1mM Rhodamine-6-2' dUTP
turns the	25 μl	chimera
entire rxn. mix	3.5 μl	TdT (10 Units)
purple.	1.5 μl	dH <sub>2</sub> O
	40 μl	total

incubate 50' at 37°C  
- Promega catalog recommends longer incubation

SIGNATURE

002261

PURPOSE Rhodamine-labeling of ALS2/D

NOTES cont 1-16-97 (p16)

DATE 1-16-97

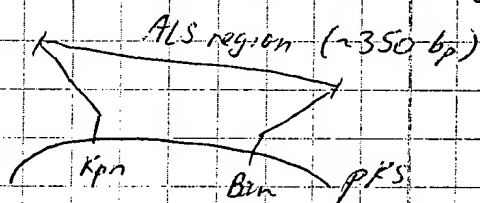
Rhodamine-labeling (cont) -

After incubation, I added (on ice): 4  $\mu$ L 0.2M EDTA pH8  
 to each of the four tubes 5  $\mu$ L 4M LiCl  
 150  $\mu$ L 100% EtOH (-20°C)  
 allowed to ppt. O/N at -80°C after inverting to mix.

Footprinting / Strand transfer assay - (preparation)

We will want to 3' end label the chimeras to footprint them and also to demonstrate binding between DNAs.

We will want to label the ALS region that I subcloned into pRS.



We will cut w/ Bam and fill in, followed by cleavage at Kpn. Unfortunately Kpn is a 3' overhang and can't be filled, so we can only footprint one strand.

I ordered  $\gamma$ -dATP and  $\alpha$ -dATP for postcut fill in of the BamHI overhang.

Protoplasting (preparation) -

I made 400 mL Protoplast isolation media (PIM)

0.25 M mannitol (45g)

50 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (7.35g)

10 mM NaOAc (0.82g)

400

18g

2.94 g

0.328g

pH'd, brought up to  
 volume, filtered  
 sterilized.

pH 5.8

1L

002262

SIGNATURE

Peter Kipp



## PURPOSE

Protoplasting - ALS chimera Elpr.  
Quantitate GFP plasmids  
Prepare Rhodamine labeled chimeras

## NOTES

DATE

1-17-97

Protoplasting - By ~~staining~~ digesting the cell walls from NT-1 cells allows the cells to more efficiently take up foreign DNA after a heat shock or electroporation.

I began stirring the enzyme mixture in 50 mL P1M  
- 150 mg Cellulase 3, 10 mg Pectolyase Y23  
- in a sterile, rimmed 100 mL beaker.

I began pelleting the cells (innoc. 1-15)  
- allowed ~75 mL total volume of cells to settle at RT  
in the TC hood, in two sterile 50 mL falcon tubes.

For complete protocol, see blue book

- after 20' of settling, the packed cell volume was ~8 mL,  
roughly 4 mL in each tube.

- I added ~6 mL of dense cells to each tube (innoc 1-8)  
- this increases cell number, but also adds many  
cell clumps to the mixture. This lowers the % of  
single cells, but increases the total cell count.  
The major concern is that I will not have enough PPLS  
to use 2 million / Elpr. By increasing the cells, I will  
have extra, so I can use  $> 2 \times 10^6$  partially protoplasted  
cells.

- I allowed the cells to settle 15' longer.  
- I removed and discarded the conditioned media  
- it is difficult to filter the cond media due to  
cell clumps, so I have been used uncond NT-1 liq.

- washed cells by adding 40 mL P1M to each tube and  
mixing ~5x.

002263

SIGNATURE

## PURPOSE

Protoplasting NT-1  
Rhodamine-labeled chimera prep.

## NOTES

Can't 1-17-97 (pg 18)

DATE

1-17-97

Protoplasting (cont.):

1 aliquoted the PIM + Enz mix into Corex tubes.

1 spun the PIM + Enz @ 10,000 rpm for 10' at 10°C (SS-34)

1 spun the NT-1 cells @ 700 rpm in the table top Sorvall for 5'.

Removed super from NT-1 (not completely pelleted)

- washed again in 40 ml PIM each, inverted to mix
- spun cells as before

- removed PIM + Enz (small pellets roughly the area of a pencil eraser) aliquoted into a 50 ml Falcon tube.

Removed super from NT-1 cells (again not completely pelleted)

- added ~~20~~ ~15 ml PIM + Enz to each ~6 ml pellet
- mixed by inversion
- aliquoted ~15 ml resuspension to 3 petri dishes, wrapped in parafilm, covered in foil
- began shaking @ 40 rpm at 11:20 AM, at RT.

Rhodamine labeled chimeras -

1 spun the 4 tubes at 14,000 x g for 25 min. at RT in p.fuge.

- the pellets were bright purple making them easy to ID
- removed super, washed in 100  $\mu$ L 70% EtOH (-20°C)
- spun 3' at 14xg
- removed super, pulse spun, removed all liq. that I could.
- inverted tubes on fresh paper towel, covered w/ paper to prevent direct illumination
- allowed to air dry while I took UV readings (p 20)

- resusp. each chimera in 100  $\mu$ L dH<sub>2</sub>O  $\Rightarrow$  ~60 ng/ $\mu$ L

- same [ ] as unlabeled chimeras.

- placed in -80°C ("CHIMERAS" Box)

SIGNATURE

002264

NAME P. Kipp

EXP. NO. ASSAY

20

PURPOSE

Protoplasting NT-1 / Elp'n  
Rhodamine labeled chimeras  
UV (A260) of pGEM GFPs.

NOTES

Cont 1-17-97 (p19)

DATE

1-17-97

pGEM-GFPs 2/100

1-17-97

SAMPLE	A280	A260	280/260	260/280	
1.0000	0.1036	0.2072	0.5289	1.8909	gen 1
2.0000	0.0982	0.1845	0.5322	1.8790	gen 2
3.0000	0.0857	0.1611	0.5319	1.8800	gen 3
4.0000	0.0823	0.1675	0.5251	1.9043	gen Δ1
5.0000	0.0741	0.1380	0.5141	1.9453	gen Δ2
6.0000	0.0700	0.1446	0.5181	1.9264	gen Δ3
7.0000	0.1032	0.1940	0.5323	1.8787	gen stop1
8.0000	0.0960	0.1825	0.5260	1.9013	gen stop2
9.0000	0.1108	0.2175	0.5062	1.8310	gen stop3

A260/280 ratios of ~1.9 indicate possible presence of RNA.  
The actual [I] of the plasmids is 2.5x the A260.

Protoplasting - at 2:45 PM (~3:30 after start) I took an aliquot of the cells and checked the progress of the digestion.

As expected, there were many clumps, which were partially digested. There were also a good number of single cells that were completely round, as expected for protoplasts.

I moved the cell + enz mix to a 50 mL Falcon tube.

- I took a small aliquot to observe on the microscope to see if the enzymes have a red fluorescence.

I added ~10 mL of P1M and spun the PPS at 700g for 5'

- incomplete pelleting (OK)
- removed super w/ vacuum apparatus
- added 40 mL P1M, added gently, inverted gently to mix
- spun as before, removed super, added 40 mL P1M
- <sup>spin</sup> washed again, resusp. cells in ~25 mL POR
- counted on hemacytometer ((grid 1 + grid 2) ×  $\frac{1 \times 10^4}{2}$ )
- indicated ~1 million cells/mL.

SIGNATURE

002265

NAME P. Kipp

EXP. NO. ASSAY

21

PURPOSE

Protoplasting NT-1 Eip in ALS  
chimeras  
UV microscopy

NOTES

cont 1-17-97 (p20)

DATE

1-17-97

Protoplasting - The cell count is only a rough estimate as many of the cells are in clumps, and the number is difficult to gauge. Perhaps 35%-50% of the counted cells are single cells.

Spin cells as before, removed supernatant.  
- resusp. in 10 mL POR

While the cells spin, I took out 5 chimeras: ALS 1-3  
- and placed 6 0.4 cuvettes on ice ALS D  
ALS 2 + Rhod. (dark)

After resuspending the cells in POR, I placed them on ice.  
- after ~5' on ice, I aliquoted 1 mL of PPLS into each cuvette, and then added 25  $\mu$ L 40  $\mu$ L of the chimeras to the appropriate cuvette, leaving one cuvette w/ just cells.

Eip in - BioRad

inverted cuvettes 2x to mix

Capacitance extender at 250

Voltage

0.25

to ~ 6 ms

In hood, I moved the Eip'd cells to 7.5 mL POM in a 2 in petri dish.

- I made slides of: ALS-2  
ALS-2 + Rhod  
Eip'd only

wrapped petri dishes, placed them in the dark.

Fluorescence Microscopy - I checked the slides on the oscilloscope and both UV settings. Took 3 photos of semi-protoplasted cells.

Red channel - "Eip'd only" and "ALS-2" are completely black  
- 1 photo, 5 min exposure

002266

ALS-2 + Rhodamine is very bright red.

SIGNATURE

PURPOSE

UV microscopy of ALS-2Rhod  
in NT-1 cells

NOTES

1-17-97 con't (p21)

DATE

1-17-97

Microscopy con't

ALS 2+Rhod. was very bright. The fluorescence was entirely nuclear and the background also appeared red. Unpenetrated cells appeared completely black w/ no hint of red.

- took various photos ~ 1 min exposures
- checked cells on fluorescein channel
- Many of the cells appear green under these conditions for ALS-2 and ALS 2+Rhod.
- took one photo of background green fluorescence

film - Kodak Ektachrome 400 Elite

Electroporated Cells - I took a 15  $\mu$ l aliquot of each of the experiments and spotted it on 50 ppb glean NT-1 media. I am curious to see if any cells can regenerate without recovery. I also spotted some cells on NT.

I dropped the film at Photo USA.

Peter Kipp

begin 1-18-97 Large-scale NT-1 cell growth Elpid cells  
tobacco explants  
media, DNA xt buffer

NT-1 Cells - I autoclaved 3 500 mL flasks to grow NT-1 cells.

I added 125 mL NT-1 media to each flask, then inoculated with 5, 6 or 7 mL of a very low density NT-1 cell culture (1-17)

I placed the flasks in the "growth" room shaking at 105 rpm

002267

SIGNATURE

PURPOSE

Large scale NT-1 Eipid Cells  
tobacco explants Slides  
media

NOTES

1-18-97 cont (p22)

DATE

1-18-97

Tobacco Explants - I cut ~5 leaves and stems into pieces of various sizes and shapes and placed them on a callus induction media - LC-1 (for potato). The pieces ranged in the degree of wounding. The plates were wrapped and placed in the growth room.

### Electroporated Cells -

I began shaking the cells at 40 rpm, in the dark.

Made reagents: DNA extraction buffer: 1% Sarkosyl  
(genomic DNA) 0.8M NaCl

made 100 mL

0.022M EDTA (pH 8.0)

0.22M Tris-HCl (pH 7.8)

0.8% CTAB

0.14M Mannitol

$$0.1L \times \frac{0.8 \text{ moles}}{1L} \times \frac{58.44g}{1 \text{ mole}} = 4.675g \text{ NaCl}$$

$$0.1L \times \frac{0.022 \text{ moles}}{1L} \times \frac{1L}{0.5 \text{ moles}} = 0.0044L$$

4.4 mL 0.5M EDTA

1g Sarkosyl

$$0.1L \times \frac{0.22 \text{ moles Tris-HCl}}{1 \text{ mole}} = 0.022L$$

22 mL 1M Tris pH 7.8

0.8g CTAB.

$$0.1L \times \frac{0.14 \text{ moles}}{1L} \times \frac{182.17g \text{ mannitol}}{1 \text{ mole}} = 255g \text{ mannitol}$$

began stirring w/ gentle heat - continued stirring w/o heat.

YEN-B (low salt bacterial media): 0.8% Nutrient Broth  
0.75% yeast extract

made 500 mL → 4g nutrient broth  
3.75g yeast extract.

autoclaved 25'

Scanned selected NT-1 micrographs into the computer  
via adobe photoshop.

002268

SIGNATURE

Peter Kipp



PURPOSE

Plate Eipol cells (check Fluorescence)  
Inoculate ALS1-3A#3  
DNA xt buffer

NOTES

DATE

1-19-97

Plate NT-1 Electroporated cells onto selective media -

I plated ~4ml of the cells onto large plates w/ 30 ppb gleam.  
I left the plates open in the hood to dry.

The remainder of the cells were returned to the shaker.

- I took a small aliquot of ALS2+Rhod to observe the fluorescence.

- the cells were still very red, roughly 48 hrs after being electroporated.

ALS1-3A #3 - I inoculated a ~50 mL YENB + 100 µg/mL AMP with a single colony from the ALS1-3A #3 plate, shaking @ 37°C.

The ALS1-3A#3 plasmid will be used in the footprinting and strand transfer assays. This plasmid has the SuRA locus; sequenced 12-14 (p130 IME book 2).

I brought the volume of the DNA extraction buffer to 100 mL and aliquoted it into 2 x 50 mL Falcon tubes.

This buffer had intentionally been left ON w/ continuous stirring.

002269

SIGNATURE

Peter Kipp

PURPOSE

50mL plasmid prep ALS1-3A #3  
genomic RT-1 cell DNA isol.  
- PCR screen  
plate remainder of Elp'd cells.

NOTES

DATE

1-20-97

Genomic DNA Isol. -

I sampled two calli that are both apparently regenerating on  
30 ppb gleeam; ALS 2-3  
ALS 1-1

The calli look roughly the same, however 2-3 is a lighter color.

When I collected the callus, 1-1 was very firm, almost brittle,  
while 2-3 was soft and moist. I collected ~100µL of each  
tissue. I placed the tubes on ice.

### Plant Genomic DNA Isolation

- 1) Grind young leaf tissue with a mortar and pestle under liquid N<sub>2</sub>.
- 2) Add frozen leaf powder to a tube containing pre-warmed (65°C) DNA isolation buffer and mix with gentle inversion (use 2 ml of buffer per gram of leaf tissue).
- 3) Quickly add an equal volume of CHCl<sub>3</sub>/isoamyl-OH (24:1) and mix by gentle inversion (Note: Vent the tube periodically since a build up of pressure can occur).
- 4) Incubate ~~at~~ this mixture at 65°C for 10 min with occasional inversion.
- 5) Centrifuge samples 5 min to separate phases.
- 6) Remove aqueous layer to a new tube and add an equal volume of isopropanol, and mix.
- 7) DNA can be either spooled-out or centrifuged.
- 8) Wash sample with 70% EtOH, and dry briefly.
- 9) Resuspend in TE (pH 8.0)

Note: This DNA is pure enough for use in PCR reactions and crude Southern hybridizations. If more critical experiments are planned, RNase treat the samples after step nine to insure that all detectable RNA is removed, and quantitate. DNA isolated with this method is pure enough for use in the construction of genomic libraries. This procedure has successfully been scaled up (several grams of tissue) and scaled down (milligrams of tissue).

changes - ground in epitate with blue pestle and did not  
freeze in liquid nitrogen

used 100 µL XT buffer (no β-me)

002270

SIGNATURE

P. Kipp



PURPOSE

50 mL plasmid prep  
genomic DNA isol. 3 PCR, isol. frags. Cont 1-20-97 (p25)  
plate remainder of cells.

NOTES

DATE

1-20-97

50 mL plasmid prep - I began the plasmid prep while the genomic DNA samples were incubating -  
- spun in 2 30 mL Corex tubes 10 x 10', 10'.

For protocol, see blue book -

modified alkaline lysis w/ isopropanol ppt.  
followed by Rnase, protease/prutk,  $\phi$ -08/ $\text{CHCl}_3$

The cell pellets were dense -

I used 2x the volumes of sol'n II and sol'n III  
(2.4 mL II to each, 1.8 mL III to each)

Combined into 1 tube after lysis, added 500  $\mu\text{L}$   $\text{CHCl}_3$   
to stabilize interface.

- C'fuge 10' at 12,000 x g.
- moved super to new tube  $\rightarrow$  filtered through minicloth
- add 0.6x volumes of isopropanol
- ppt. at RT 15'
- spin 20' at 12,000 x g

while the plasmid prep was ppting, I spun out the chromosomal DNA which was ppting in ~~DNA~~ isopropanol. I washed the chromosomal pellets once in 200  $\mu\text{L}$  cold 70% EtOH. Spin 5 min, removed super - air dried on bench top.

I resuspended the plasmid pellet in 300  $\mu\text{L}$   $\text{dH}_2\text{O}$ , after drying it in the speed-vac.

- began Rnase treatment, added 4  $\mu\text{L}$  10 mg/mL Rnase  $\rightarrow$  37°C.

I resuspended each of the genomic DNA samples in 75 mL 65°C  $\text{dH}_2\text{O}$ . They went into sol'n after ~5 min at 65°C. Mixed by flipping tube; did not vortex or pipet up & down.

002271

SIGNATURE

P. Kipp

NAME <b>P. Kipp</b>	EXP. NO.	ASSAY
PURPOSE 50 mL plasmid prep ALS Genomic DNA PCR, Isol. frags plate Elp'd cells	NOTES cont 1-20-97 (pg 26)	

27

DATE  
1-20-97

### Genomic PCR of ALS 1-1 and ALS 2-3 -

+ control - I took a 4  $\mu$ L aliquot of the ALS1-3A #3 bacterial cells and mixed them w/ 21  $\mu$ L dH<sub>2</sub>O to use as a + control.

25  $\mu$ L DNA (diluted bacteria)  $\rightarrow$  100  $\mu$ L rxn.

16 $\mu$ L	1.25 mM dNTPs	56
10 $\mu$ L	10 x PCR	35
6 $\mu$ L	25 mM MgCl <sub>2</sub>	21
1 $\mu$ L	primer 1 (ALS 1)	3.5
1 $\mu$ L	primer 2 (ALS 3)	3.5
1 $\mu$ L	Taq polymerase	3.5
40 $\mu$ L	dH <sub>2</sub> O	140
75 $\mu$ L (+ 25 $\mu$ L DNA)		

Added 75  $\mu$ L premix to each DNA, overlaid w/ oil.

92°C 1'  
62°C 1' x 25, linked to file 7 (4°C soak)  
72°C 1'

Began at noon

### ALS Chimera Electroporated Cells -

I plated the remainder of the cells onto NT-1 + 30 ppb glean. ALS 1, ALS 2, ALS 3 and ALS D were plated on large plates, all 4 mL. Elp'd only and ALS 2-F were plated onto smaller plates - 2 mL volumes.

After plating the cells, I added NT-1 liquid media + 30 ppb glean to each plate (3 mL) and picked up ~~and~~ leftover cells.

any. 002272

SIGNATURE  
P. Kipp

PURPOSE

50mL plasmid prep

ALS genomic PCR, isolate PCR frag.  
transfer cells

NOTES

1-20-97 cont (pg 27)

DATE

1-20-97

Transfer of Elpid Cells - After adding NT-1 media to each dish, I moved the cells to two adjacent wells of a 12 well plate. For the "Elpid only" and "2-F" the effective concentration of glean is not 30 ppb because I added 3mL to an already existing ~2mL - for the others there was no liquid left. Thus, for "2-F" and "Elpid only", the glean conc. is roughly 20 ppb ( $30 \times \frac{2}{3}$ ). I wrapped the culture dish and placed it on the shaker.

ALS genomic DNA PCR - I poured a 0.8% TAE gel for direct isol.

The PCR ended ~2:15. I performed a quick  $\text{CHCl}_3$  xt to remove mineral oil from the reactions.

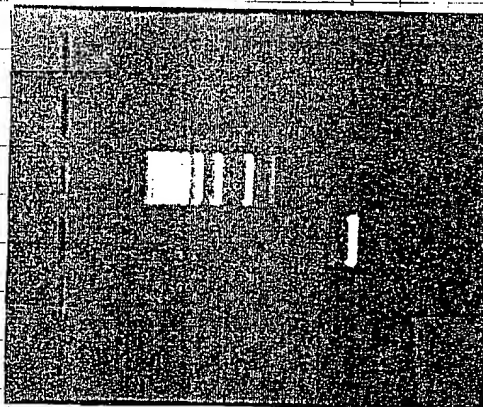
- loaded 40  $\mu\text{L}$  1-1, 2-3, 25  $\mu\text{L}$  plasmid

Genomic PCR

ALS 2-3

ALS 1-1

@ 62°C.



2-3 minimal amplification.

ALS plasmid

1-1 isol. frag  
(very intense)

I stored the ALS 1-1 band at 4°C.

I set up the PCR again. I carefully mixed and heated the ALS 2-3 DNA to 65°C before taking the 25  $\mu\text{L}$  aliquot. In addition, I set up a fourth reaction using 25  $\mu\text{L}$  of the first 2-3 PCR attempt to see if I can amplify the region from it.

- not enough starting material?
- did the rxn. fail for some other reason?

002273

SIGNATURE

P. Kipp

PURPOSE

ALS genomic PCR, isol. bands  
50 mL plasmid prep.

NOTES

i-20-97 cont (p 28)

DATE

1-20-97

Genomic PCR - The components were made into a premix as shown on pg 27, using 4.5 as the multiplier (not shown).  
- identical cycling cond  
- began at 5 PM.

50 mL ALS SuRA plasmid prep - (@ 37°C ~ 4hrs + Rnase)

The plasmid prep slipped my mind. I hope the DNA isn't degraded.

protease treatment: added 3.75  $\mu$ L 1M Tris pH 7.8  
7.5  $\mu$ L 0.5M EDTA pH 8  
@ 37°C, 5:25 PM. 9  $\mu$ L 20% SDS  
45  $\mu$ L dH<sub>2</sub>O  
9  $\mu$ L protease (20mg/mL)  
~75  $\mu$ L total

At 6:30 PM, I divided the sample into 2 epitubes and  $\phi$ -OH/ $\text{CHCl}_3$  extracted it. Spun 5' @ 12,000 x g to resolve phases.

- saved super to new tube,  $\text{CHCl}_3$  x 1
- spun, moved to new tube
- vol = ~150  $\mu$ L; added 50  $\mu$ L 3M NaOAc and 450  $\mu$ L 100% EtOH.
- placed @ -80°C o/n.

poured 0.8% TAE gel during c'fuge -

cleaned PCR reactions as before w/  $\text{CHCl}_3$   
loaded 40  $\mu$ L, order different: ALS 2-3 reamp.

Genomic PCR of ALS region  
@ 62°C  
Isolated both ALS 2-3 bands.  
SuRA plasmid  
Reamplification 1-1  
may have introduced PCR errors.

Saved gel slices @ 4°C o/n.

ALS 2-3

SIGNATURE

P. Kipp

002274

PURPOSE

gel purification of ALS frags  
run ALS1-3A#3  
streak gfps.

NOTES

DATE

1-21-97

Note - Last night I sent 9 plasmids to Naomi at Kimeragen:

p gem gfp 1	p gem gfp $\Delta 1$	p gem gfp S1
p gem gfp 2	" gfp $\Delta 2$	" gfp S2
p gem gfp 3	" gfp $\Delta 3$	" gfp S3

I decided to have these sequenced and then I will subclone the correct fragment into 1BT210.1. We don't have primers for convenient sequencing of 210.1; pGem uses T7 and SP6.

I tried ~2  $\mu$ g of each plasmid and sent 15  $\mu$ l (15 pmol) T7 and SP6 primers (see [ ] data of plasmids, pg 20).

Prep-a-Gene Purification - I used the prep-a-gene kit (for protocol see blue book) to purify the 1-1, and 2-3 bands.

The gel slices were melted @ 55°C.

The matrix was very thick, even after extensive vtx.

I did 2 x 10  $\mu$ l elutions using dH<sub>2</sub>O.

ALS fragment A260's

SAMPLE	A280	A260	280/260	260/280	1-21-97
1.0000	0.0160	0.0173	0.9261	1.0798	2 $\mu$ l 1-1 PCR
2.0000	0.0235	0.0289	0.8144	1.2279	2 $\mu$ l 2-3 PCR
3.0000	0.0290	0.0361	0.8040	1.2438	2 $\mu$ l 2-3 PCR

The A260/280 ratios are very low. I decided to quantitate the bands by comparing them to known quantities of DNA on an EtBr gel.

002275

SIGNATURE

P. Kipp

NAME P. Kipp

NOTES

PURPOSE

1. gel purification of ALS fragments  
 - quantitate  
 streak GFPs  
 run plasmid

1-21-97 cont (p30)

DATE

1-21-97

I poured a 0.8% TBE gel; I loaded 3  $\mu$ L (1.5  $\mu$ g) of the 8tst gene  
 Kb ladder and 2  $\mu$ L of each gel purified  
 fragment - also 1.5  $\mu$ L each ALS-3A#3 digest.

Quantification of  
 PCR products  
 from ALS genomic  
 PCR

- ALS-3A#3  
 plasmid.

ALS-3A#3 B

ALS-3A#3 A

2-3 reamp.

2-3

1-1

The gel is very overexposed to visualize the 500 bp band  
 below the EtBr front.

750 bp band 2.1% of total } estimate 2-3 reamplification  
 500 bp band 1.9% of total } band intermediate of these two

2% of 1.5  $\mu$ g = 30 ng in 2  $\mu$ L  $\rightarrow$  15 ng/ $\mu$ L

The other two bands are much less - too faint to use, I will need  
 to repurify them.

GFP clones - I streaked cells onto LBA for all 9 of  
 the plasmids that I sent to have sequenced.

I made 300 mL 6% sequencing mix: 126 g Urea  
 dissolved in ~ 150 mL  $\text{dH}_2\text{O}$  (combined volume).  
 stirred w/ heat.

- added 36 mL 5x TBE
- added 37.5 mL 40% acryl (19:1)
- brought vol. to 300, filter sterilized
- stored @ RT in foil

002276

SIGNATURE

Peter Kipp



PURPOSE

PCR Frags. - sequencing / isol.

NOTES

1-22-97

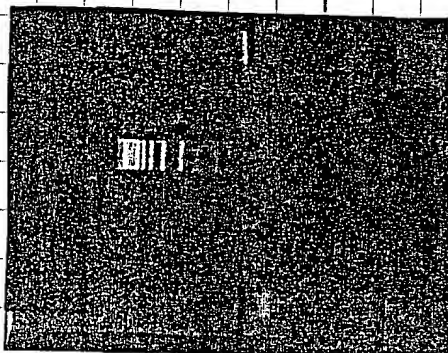
DATE

1-22-97

As the yields were low for the ALS 1-1 and ALS 2-3 PCR frags, I decided to run ALS 1-1 again and to isolate SuRA.

0.8% TAE gel-

Isolation of ALS  
PCR frags. for  
sequencing



SuRA (ALS plasmid)

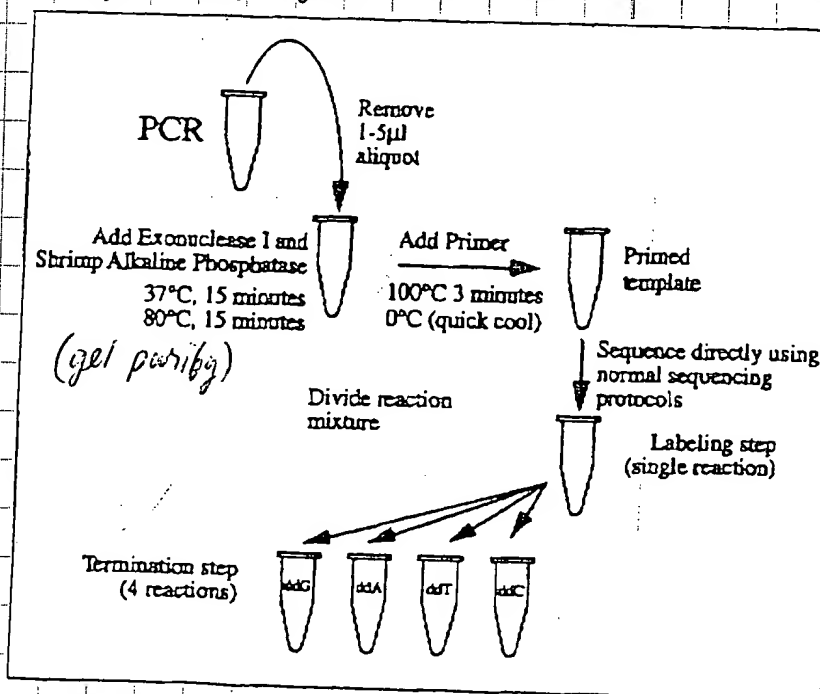
ALSP is ~ 3x as  
intense as ALS 1-1

ALS 1-1

I cut the two bands and purified them using the gleen clean kit. Spc matrix, eluted  $2 \times 10 \mu\text{L}$  dH<sub>2</sub>O.

I will sequence: ALS 1-1  
ALS 2-3 (re-PCR)  
ALS SuRA

Direct sequencing of PCR products: Amersham technical



002277

SIGNATURE

P. Kipp

NAME P. Kipp

EXP. NO. ASSAY

33

PURPOSE Sequencing PCR &amp; gtps

NOTES

1-22-97 cont (pg 32)

DATE

1-22-97

Sequencing GFP plasmids -

I just spoke to Naomi @ Kimeragen

- I didn't send enough primer, she needed 5 pmol / rxn.
- they have T7 and SP6 primers, but they are different.
- the T7 primer diverges at the 3' end and can't be used on our template, but the SP6 can.

- Naomi said that she will dilute the T7 and try it

I decided to sequence 4 GFP plasmids w/ T7, hopefully together we will have all the data we need.

I chose  $\Delta 1$ ,  $\Delta 2$  and stop 1, stop 2.I denatured ~4  $\mu$ g each in 20  $\mu$ L total using 2  $\mu$ L 2/2.- 10  $\mu$ L  $\Delta 1$ ,  $\Delta 2$ - 8  $\mu$ L S1, S2 @ 37°C

2.0

I ppt by adding 2.0  $\mu$ L 10 M NaOAc and 80  $\mu$ L EtOH.

- @ -80°C 15'

PCR Frags - I will try to use ~100 ng of each PCR frag for the sequencing.

(~100 ng)  
3  $\mu$ L ALS-P  
4  $\mu$ L ALS 2-3  
8.5  $\mu$ L ALS 1-1

I did not quantitate ALS-P and ALS 1-1, but I assumed based upon the intensities of the bands in the gel. The ALS-P was slightly more intense than ALS 2-3.

procedural notes - I set up the annealing reactions in H<sub>2</sub>O only - no 5x sequenase buffer

- heated 3 1/2' @ 100 in cycles

- cooled on ice, pulse spin.

002278

SIGNATURE

P. Kipp



1-22-97 cont (pg 33)

DATE

1-22-97

Sequencing - spun, washed and dried GFP plasmids

- resusp 7  $\mu$ L  $H_2O$  added 2  $\mu$ L 5x seq. buffer and 1  $\mu$ L T7
- annealed @ 37°C, 30'

Set up sequencing premix:

1 $\mu$ L DTT (0.1M)	
2 $\mu$ L dGTP labeling mix (1:7.5)	
8 $\mu$ L DTT	$\leftarrow 8 \times 0.6 \mu$ L $\alpha$ $S^{35}$ dATP
16 $\mu$ L labeling mix	2 $\mu$ L sequenase (1:8)
4.8 $\alpha$ $S^{35}$ dATP	

(16  $\mu$ L sequenase)  $\rightarrow$  made 20  $\mu$ L kept separate.

Pulse spin all templates, placed on ice. Set up term tubes. @ 37°C.

Started plasmids by adding  
3.5  $\mu$ L premix and 2  $\mu$ L enzyme.

Started PCR products by adding  
2  $\mu$ L 5x sequenase buffer, 3.5  $\mu$ L  
premix and 2  $\mu$ L enzyme.

Amer sham recommends a 5-10  
minute extension time - I used  
6  $\mu$ t min.

The gel pre-run for ~ 15 min  
at 1500 V.

35 gD1 start 31 term 27 stop

33 gD2 start 29 term 25 stop

30 gS1 start 26 term 21 stop

24 gS2 start 20 term 16 stop

22 1-1 18 term 12 stop

19 2-3 15 term 9 stop

13 P 12 term 3 stop

D1 D2 S1, S2, 1-1, 2-3, P

1-22

loaded as shown, w/ 5 min delay between S1 and S2 etc.

- gel ran 1:20

- ff to whatman

- dried @ 80°C, 55 min, exposed OVN.

002273

Removed GFP plates from incubator, wrapped, moved to 4°C.

SIGNATURE

Peter Kipp

Loaded GATC

ALS-PERS

1-23

Δ1

Δ2

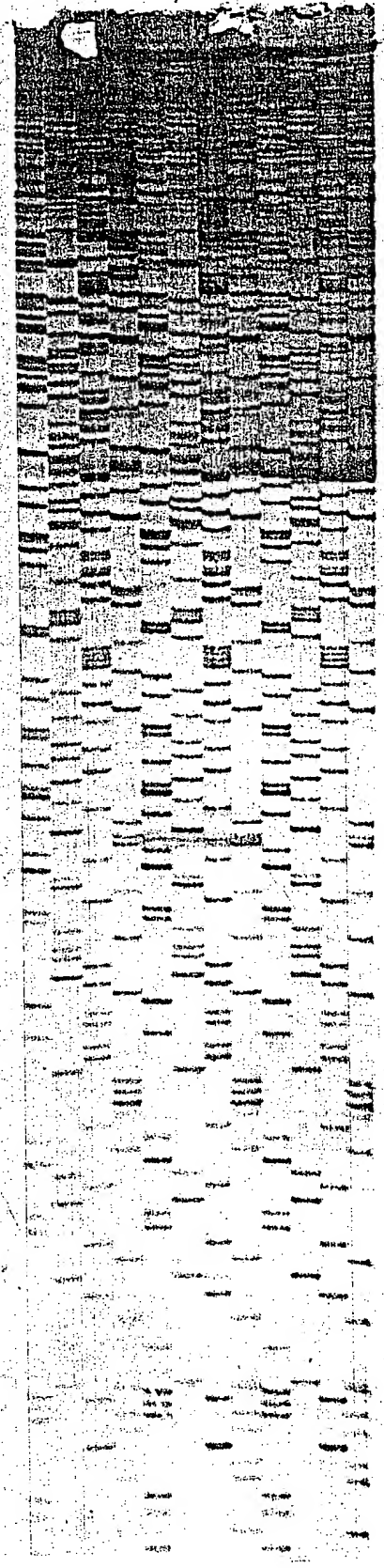
S1

S2

1.1

2.3

P



PURPOSE

Dev. seq. gel  
Isolate nuclear extracts

NOTES

1-23-97 start.

DATE

1-23-97

Developed seq. gel (facing page).

- GFPs failed, too much DNA?

- clearly the premix worked as the PCR products sequenced clearly.

Sequence Analysis - reading from ACSI

The sequences are all identical except for one ambiguity in the sequence of 2-3 at the sequence corresponding to Pro 196.

The CCA codon reads C, A, the second C is highly smeared, with bands in each of the four lanes. The photocopy shows some smearing in the first C also, which is faint, but still present on the actual film (bottom drawer, left).

The 1-3 is growing well on 50 ppb glee, so this sequence ambiguity seems an improbable coincidence.

As far as I could read, there do not appear to be any errors introduced by PCR.

Furthermore, there are no signs of the SuRB locus

- PCR @ 62°C is sufficiently selective to favor the SuRA.
- this eliminates the need to sequence the ACSI-3 C PCR subclones - I discarded them.

I spoke to Naomi at Kimeragon -

she was able to get sequence on some of the gfp plasmids that I sent, even w/ low quality quantity of primers.

GFP plasmid seq. results -

GFP 1 - one change

GFP 2 - failed

GFP 3 - perfect ✓

GFP S1/S3 - not complete

GFP S2 - perfect

S1 to AA seq

STQSALS...

S3 not as far.

will complete sequence myself w/ SP6.

← GFP S1/S3 - perfect, but incomplete

GFP S2 - failed

002281

SIGNATURE

P. Kipp

NAME P. Kipp

EXP. NO. ASSAY

PURPOSE

ALS/GFP sequencing  
Nuclear extracts

NOTES

1-23-97 cont (p35)

DATE

1-23-97

Sequencing of GFPs - Summary -

pGEM GFP 12 ✓

pGEM GFP 3 ✓

pGEM GFP stop 1/3 possibilities.

10	20	30	40
M V S K G E E L F T G V V P I L V E L D G D V N G H K F S V S G E G E G D A T Y 40			
G K L T L K F I C T T G K L P V P W P T L V T T F T Y G V Q C F S R Y P D H M K 80			
Q H D F F K S A M P E G Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L 120			
V N R I E L K G I D F K E D G N I L G H K L E Y N Y N S H N V Y I M A D K Q K N 160			
G I K V N F K I R H N I E D G S V Q L A D H Y Q Q N T P I G D G P V L L P D N H 200			
210	220	230	240
Y L S T Q S A L S K D P N E K R D H M V L L E F V T A A G I T H G M D E L Y K. 240			

GFP Coding translation

In my conversation w/ Naomi I tried to learn the sequences of the clonings - she'll check and see about authorizing it.

Also, she suggested that I sequence my PCR products from the other direction to see if the ambiguity still occurs.

ALS PCR sequencing - ALS 3 primers

I set up as before: 3  $\mu$ L ALS-P  
 4  $\mu$ L 2-3 → + 1  $\mu$ L primer 3 dH<sub>2</sub>O → 10  $\mu$ L  
 0.5  $\mu$ L 1-1 100 °C 3'

Placed samples on ice, (poured seq. gel. before starting)

Made premix as for ds templates - 1  $\mu$ L DTT

0.6  $\mu$ L  $\alpha$ S x 4

added 2  $\mu$ L 5x Sequenase 2  $\mu$ L labeling mix  
 buffer separately as on 1-22 (2  $\mu$ L enz 1:8 separate)

002282

SIGNATURE

P. Kipp

NAME

P. Kipp

PURPOSE

ALS seq.  
Nuclear extracts

NOTES

1-23-97 Cont (p36)

DATE

1-23-97

ALS sequencing -

Runs. as shown. I loaded the first run into the gel at 1:15 PM, @ 1500 V, after a 15' pre-run.

Since I need to read ~ 300 bases into the sequence, I will run the gel under the XC reaches the bottom.

I loaded 2.3 ALS1 primer again @ 2:30 to run further.

24 1.1 St. 30 term 14 stop

22 2.3 St 18 term 12

21 P St 17 term 11

GATC

3.25 µl ea.

1115

2:40

4 PM

2.3 1.1 2.3 P3 1.1 2.3 P3

1 3 3 1-23

The gel is running slowly - I changed the buffer to fresh 0.5x TBE.

Nuclear Extracts -

The cells have not grown sufficiently. We will look again on Friday.

GM made a slide of the ALS2-F cells that are shaking in liquid media. They are still red (6 days post.).

The Elp'd materials are beginning to show signs of recovery. The ALS1-#2 plate has a few very small bumps, as do the ALS2 plates.

ALS Elp'd and ALS3-91 has spores beginning to form

- I tried to remove the contaminated areas, but I don't know if I was completely successful.

I loaded the 2nd set of sequences w/ primer 3 at 4 PM.

- gel ran very slowly → 2:30 to get the Bp6 close to the bottom.

- ff, dried @ 80°C

- set up on film. O/N.

002283

SIGNATURE

P. Kipp

NAME

P. Kipp

PURPOSE

GFP cloning  
ALS sequence interpretations

NOTES

1-24-97 start

DATE

1-24-97

Note: Last night I set up digests for the GFP subclonings as well as ALS.

GFP - I digested 2.75  $\mu$ L of each plasmid (GFP 3,  $\Delta 2$ , S1) w/ Sac I, buffer J  $\rightarrow$  total vol. = 25  $\mu$ L.

ALS - I digested 4  $\mu$ L of the 2-3 PCR product w/ Kpn, buffer J - total = 25  $\mu$ L.  
at 37°C.

After ~90 min, these digests were  $\phi$ -OH/CHCl<sub>3</sub> and ppt. (100  $\mu$ L vol.) by + 3M NaOAc / EtOH. AT -80°C 15'.

- spun 15, washed, dried

- resusp. 26  $\mu$ L dH<sub>2</sub>O for plasmids, 17 for PCR

- digested w/ Nco I (plasmids) in buffer D  
digested w/ Bant I (ALS PCR) in buffer E.

- these digests incubated ~90 min @ 37°C, stored @ 4°C O/N.

(1-24-97)

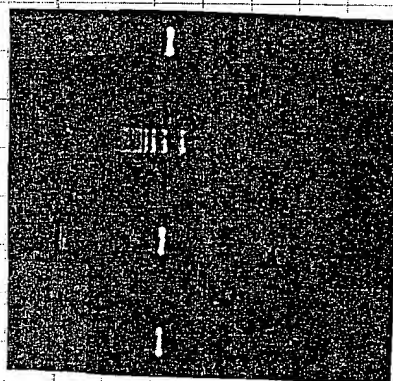
Run digests of GFPs on 0.8% TAE:

Band Isol. of GFP (Nco/Sac)  
fragments to clone into  
IBT 210.1

I purified these fragments using the Blue-Clean kit,

- 5  $\mu$ L matrix

- 2 x 10  $\mu$ L elutions (dH<sub>2</sub>O)



GFP step 1

GFP  $\Delta 2$ 

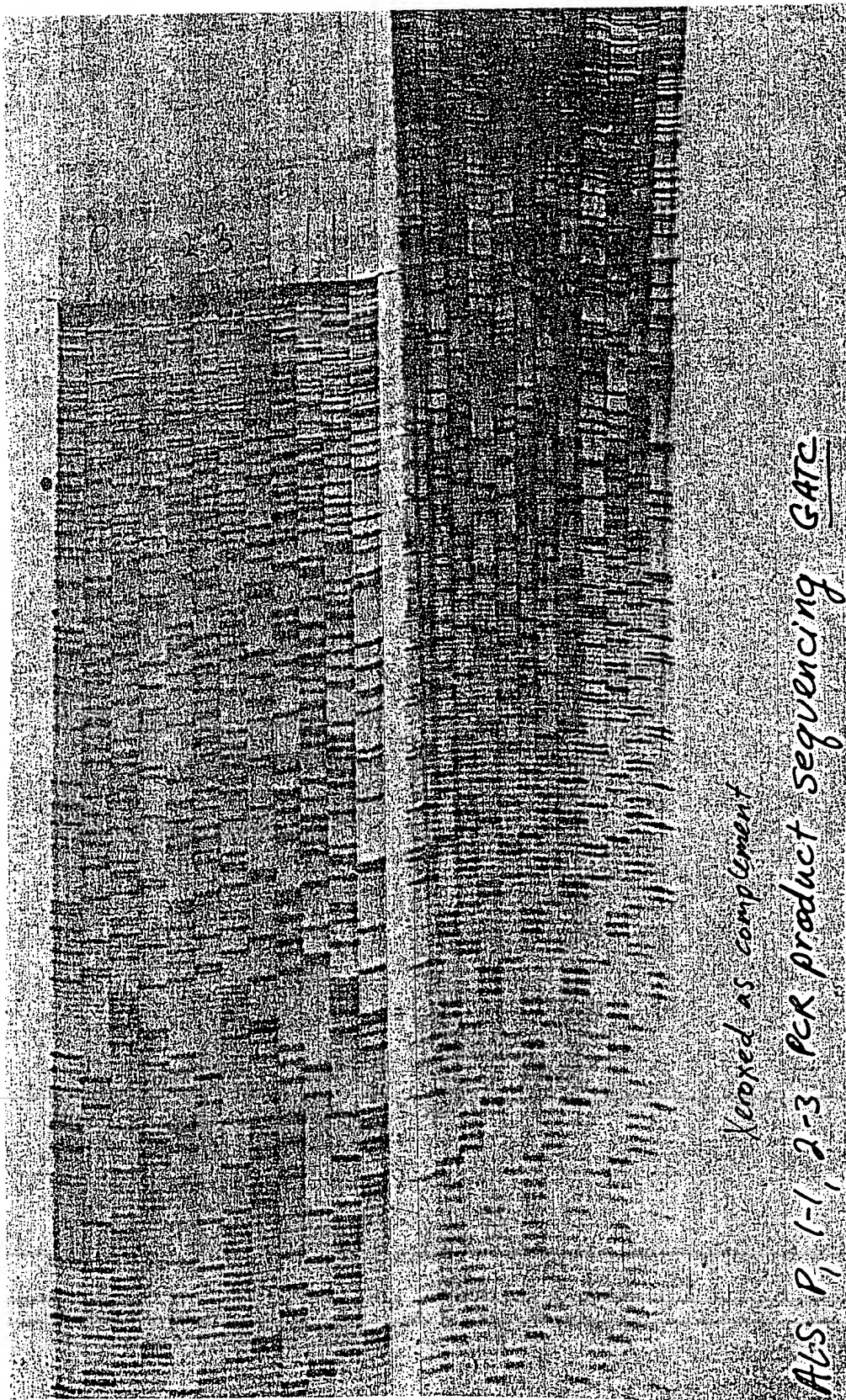
GFP #3

002284

SIGNATURE

P. Kipp





NAME P. Kipp

PURPOSE

GFP cloning  
ALS seq. interpret.  
Nuclear extracts

NOTES

1-24-97 (cont p 38)

DATE

1-24-97

GFP / ALS cloning - 1 set up 4 ligations

3 for GFPs : 1  $\mu$ L 210.1 N/S + 1.5  $\mu$ L GFP N/S  $\rightarrow$  10  
 " + 1.5  $\mu$ L GFP  $\Delta$ 2 N/S  $\rightarrow$  10  
 " + 1.5  $\mu$ L GFP stop 1  $\rightarrow$

each ligation used 1  $\mu$ L 10x promega ligase buffer and ~0.5  $\mu$ L promega enz.

1 for ALS: 1  $\mu$ L PKS B/K + 2  $\mu$ L SuRA PCR B/K  $\rightarrow$  10

The digest was used directly after 15' at 65°C to destroy the enzyme.

The ligations incubated @ RT beginning at 1:30 PM.

ALS sequencing - (photocopy opposite pg)

The sequences ran very cleanly, but the area of interest is at the very top of the gel - and it is not clear on the photocopy.

2-3

The "sequence clearly shows CCA in this direction w/ no ambiguity - at least at this level of resolution.

I did not Xerox the run of the 2-3 sequence w/ ACS-1. It shows the same ambiguity as previously described.

I will have to have these products sequenced.

Nuclear extracts - The cells still haven't grown very much.

Rrag set up larger scale immocultures - he immocultured 20 mL of the culture into 400 mL NT-1 liquid media.

- he set up two flasks, each w/ 400 mL.

002286

SIGNATURE

P. Kipp



PURPOSE

ALS Chimeras

NOTES

1-24-97 (cont pg 39)

DATE

1-24-97

Naomi provided us w/ the sequences of the ALS Chimeras.

#1 is the Pro → Gln

#2 is the Pro → Leu

Phenotypic data suggests that the absence of Pro 196 confers SU resistance, as ALS 2-3 is growing robustly on 50 ppb.

## ALS-1

T G C G C G guccaguucaCGTTGcauccuacuaT

T

T

T

T

→ Pro → Gln

TCGCGC-3' 5' -CAGGTCAAGTGCAACGTAGGATGATT

## ALS-1DNA

T G C G C GGTCCAGTTCACGTTGCATGGTACTAT

T

T

T

T

Pro → Gln

TCGCGC-3' 5' -CAGGTCAAGTGCAACGTAGGATGATT

## ALS-2

T G C G C G guccaguucaCGATGcauccuacuaT

T

T

T

T

Pro → Leu

TCGCGC-3' 5' -CAGGTCAAGTGCTACGTAGGATGATT

## ALS-3

TCGCGCG-3'

T

T

5' -TGATGT

T

T

TGCGCGCGTTGCTuauuguccaguucaCGTTGcauccuacuaucacTGACATACT

002287

SIGNATURE

P. Kipp

NAME P. Kipp

PURPOSE GFP Cloning

NOTES

1-24-97 (cont p 40)

DATE

1-24-97

GFP Cloning -

I Elp'd 2  $\mu$ L (of 10) of each of the 3 GFP and ALS ligations.

I used YEN-B as the recovery media.

- to range - 4.4 - 4.6

I allowed the cells to recover @ 37°C.

I plated 50  $\mu$ L (20mg/ml) X-gal on the LBA plate which will have the ALS cells - can't B/W select the GFPs.

I added 20  $\mu$ L 0.1M IPTG to the ALS cells while I plated them.

- to plate, I spun the cells 1' @ 9,000 rpm, removed 0.85  $\mu$ L and resusp the pellet in the remaining media. I plated all the cells

- allowed to adsorb ~10 min, placed @ 37°C o/n.

End 1-24-97

002288

SIGNATURE

Peter Kipp

NAME <b>P. Kipp</b>	EXP. NO.	ASSAY
PURPOSE <b>GFP/ALS cloning PCR screen</b>	NOTES <b>1-25-97 start</b>	
		DATE <b>1-25-97</b>

GFP Cloning / ALS 1-3 Subcloning -

There are no colonies on the ALS plate  
 - perhaps the ligation failed due to presence of restriction enzyme buffer components or glycerol.

Each GFP plate has many colonies.

PCR screen - I selected 6 colonies from each plate.  
 resusp. colonies in 20  $\mu$ L dH<sub>2</sub>O.

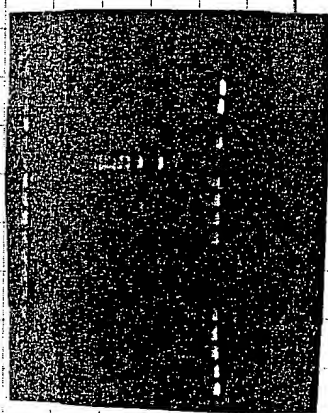
6 $\mu$ L cells / rxn :	2.5 $\mu$ L 10x	50
	4 $\mu$ L dNTPs 1.25mM	80
sited remaining	1.5 $\mu$ L MgCl <sub>2</sub> 25mM	30
cells @ RT.	0.25 $\mu$ L GFP 5' NcoI	5
	0.25 $\mu$ L GFP 3' Sac	5
	0.25 $\mu$ L Tag	5
	10, 25 $\mu$ L dH <sub>2</sub> O	205
	19.	

19  $\mu$ L premix + 6  $\mu$ L cells.  
 3 drops of oil / rxn.  
 rxn. began @ 11:45 AM.

92°C 45  
 45°C 45 x 25  
 72°C 45

powered 0.8% TBE (90mL) gel - used bottom comb

PCR screen of  
 GFP 210 putatives.  
 ID positives  
 for: GFP ✓  
 GFP  $\Delta$  ✓  
 GFP stop ✓



) GFP 1-6 (G1, G3)  
 ) GFP stop 1-6 (S5, S6)  
 ) GFP  $\Delta$  1-6 ( $\Delta$ 1,  $\Delta$ 2)  
 002289

SIGNATURE  
*Peter Kipp*

NAME P. Kipp

EXP. NO. ASSAY

43

PURPOSE GFP Cloning

NOTES

1-26-97 start/end  
1-27-97 start

DATE

1-26-97/1-27-97

210 GFP Cloning - 1 identified positives for each of the 3 GFPs.

210 GFP: # 1, 3, 4

210 GFPΔ: # 1-5

210 GFPstop: # 3-6

YEN-B + Amp

I inoculated ~4 mL cultures of: GFP 210 #1, #3  
GFP Δ210 #1, #2  
GFPstop 210 #5, 6

shaking O/N @ 37°C.

- began ~ 5:15 PM

end 1-26-97

begin 1-27-97

GFP cloning, R.E. digests, band isol, ligation  
ALS genomic PCR, DNA isol.GFP Cloning - each of the cultures grew very well.

I selected GFP Δ1, GFP 3 and GFP stop 6 to plasmid prep.

- ~2.5 mL each, via A.L.

- after III, I  $\phi$ -OH/CHCl<sub>3</sub> xt followed by CHCl<sub>3</sub>

- ppt. in EtOH @ 0°C

Genomic DNA isol. - (protocol p25)I used ~50  $\mu$ L tissue for ALS 1-1, 1-2 and 2-3.100  $\mu$ L preheated buffer, ground w/ blue pestle in epifube.

- incubated @ 65°C, ~10 min, inverted 3 times.

002290

SIGNATURE

P. Kipp

PURPOSE

GFP Cloning → Agro vectors  
ALS genomic DNA isol, PCR

NOTES

1-27-97 (cont p 43)

DATE

1-27-97

GFP Cloning - I spun the plasmid DNAs @ 14,000 xg for 10' at RT.  
- washed pellets w/ 500  $\mu$ L 70% EtOH.  
- speed vac'd, resusp. 50  $\mu$ L dH<sub>2</sub>O

Digests: I used 2.75  $\mu$ L each plasmid  
H3/EcoRI in ~~6~~ multicore buffer, total volume 25  $\mu$ L.  
began incubating at 37°C ~ 10 AM.

ALS - tobacco genomic DNA -

ppt. genomic DNA samples in by adding 100  $\mu$ L isopropanol  
- inverted ~ 8 times  
- could see the DNA ppt.  
- spun 10' @ 14,000 xg  
- washed 70% EtOH, spun again, speed vac'd 1 min.  
- resusp. each DNA in 100  $\mu$ L dH<sub>2</sub>O @ 65°C.

genomic PCR - while I made the premix, the DNAs incubated @ 65°C.

Premix - as shown on pg 27

I gently mixed the genomic DNAs and used 25  $\mu$ L for each of the 3 reactions.

The PCR cond were: 92°C 1  
58°C 1 x 27 cycles  
72°C 1  
began @ 12:15

I conducted the PCR @ 58 so that I would get a mixture of the two alleles - perhaps ALS 1-1 has the SURB locus mutated allowing it to grow on gleam.

I placed the GFP digests @ 0°C at 12:30 PM.

002291

SIGNATURE

P. Kipp

## PURPOSE

GFP Cloning, isol, ligation  
ALS-PCR, isol  
ALS-P digest

## NOTES

1-27-97 (cont p 44)

DATE

1-27-97

I set up a digest of the Sura plasmid - ALS1-3A43.

- 2.5  $\mu$ L DNA (from maxiprep)  $\rightarrow$  1  $\mu$ L BsmHI

$\rightarrow$  in buffer E, total volume 20  $\mu$ L.

begin @ 12:40 PM.

This DNA will be labeled, then cut w/ Kpn. The resulting fragment will be used in the EMSAs and other binding assays.

- Greg will label the fragment.

I loaded a 0.8% TBE gel:

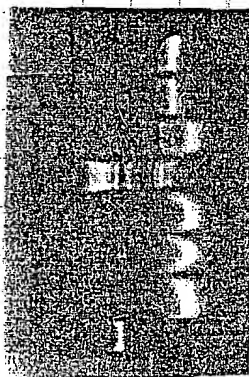
@ 130V.

2  $\mu$ L ALS1-3A43 Bsm

2  $\mu$ L each gfp digest (G, A, S)

5  $\mu$ L of each ALS PCR rxn (1-1, 1-2, 2-3)

Quick gel to check  
digestion and PCR  
amplification of  
GFPs and ALS

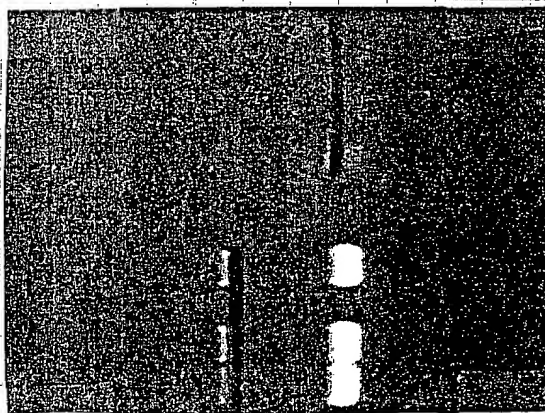


all look good

While the gel ran, I checked the PCR rxns.

I loaded a 0.8% TAE gel with each digest and 40  $\mu$ L each  
ALS PCR reaction:

Isol. of ALS PCR  
frags (G, A, S) and  
GFP 210 HIRI  
cassettes.



ALS 2-3

ALS 1-2

ALS 1-1

GFP 210 stop

GFP 210

GFP 210  $\Delta$

002292

SIGNATURE

P. Kipp



NAME *P. Kipp*

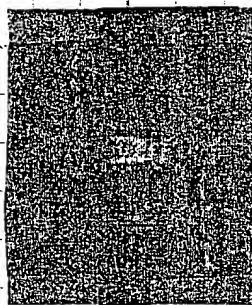
PURPOSE

*GFP Cloning  
ALS purification*

NOTES

*1-27-97 (cont p 45)*

DATE

*1-27-97**I gene-cleaned each of the fragments and eluted them in 20  $\mu$ L.**I set up GFP 100 ligations-**GFP A 100 1  $\mu$ L vect, 1.5  $\mu$ L insert**GFP 100 1  $\mu$ L vect, 1.5  $\mu$ L insert**GFP stop 100 1  $\mu$ L vect, 1.5  $\mu$ L insert**→ total 10, 0.5  $\mu$ L ligase**vector in 1BT H3JR1**@ RT, ligation began @ 7:00 AM.**I loaded 2  $\mu$ L of each of the ALS PCR fragments into 0.8% TBE:  
to quantitate the yield.**Quantitation of ALS  
PCR (@ 59) after gel  
purification.**3  $\mu$ L Kb ladder (1.5  $\mu$ g)**ALS 2-3**ALS 1-2**ALS 1-1**I estimated that each product was roughly the same as the  
two smallest bands (4% of total)*

$$0.04 \times 1.5 \mu\text{g} = 0.06 \mu\text{g} \quad 60 \mu\text{g} / 2 \mu\text{L} \text{ or } 30 \mu\text{g} / \mu\text{L}$$

*GFP Cloning - I made frozen stocks of:**GFP 210 A1**GFP 210 3**GFP 210 stop 6**I also streaked each of the six  
cultures onto an LBA plate from easy  
access to the cells.*

002293

SIGNATURE

*P. Kipp*

NAME P. KippPURPOSE ALS 1GFP sequencing  
GFP cloning

NOTES

1-27-97 (cont p 46)

DATE

1-27-97

Package to Naomi -

PCR products:

A1	}	@ 62	10 $\mu$ L	ALS 1-1
A2			5 $\mu$ L	ALS 2-3
B1	}	@ 58	5 $\mu$ L	ALS 1-1
B2			5 $\mu$ L	ALS 1-2
B3			5 $\mu$ L	ALS 1-1
ALS-P3		62	4 $\mu$ L	ALS 13A & 3

dried PCR products down in speed vac, along w/ 1  $\mu$ g stop 1 and stop 3, 2  $\mu$ L each.

1 sent 15  $\mu$ L SP6 primers

1 sent 500  $\mu$ L ALS-1 and ALS-3 primers

GFP Cloning - @ 11 PM, 1  $\mu$ L each ligation into DH5 $\alpha$  by Elpin.  $\mu$ C = 4.4 each.

Recovery @ 37°C, 60', plated entire volume on LB+Kan  
~ 12:15 AM. O/N at 37°C.

002294

SIGNATURE

P. Kipp



NAME P. Kipp

EXP. NO. ASSAY

PURPOSE

GFP Cloning  
Package to Kimeragen

NOTES

1-28-97 start

DATE

1-28-97

Sent package to Naomi - priority first, will arrive Tues 8 AM

The colonies are growing, but aren't large enough to pick.

@ 2 PM, I selected 6 colonies from each of the plates  $\rightarrow$  20  $\mu$ l H<sub>2</sub>OPCR Screen - standard cond; 6  $\mu$ l cells, 25  $\mu$ l rxn.I used GFP 5' and Sac 3' to amplify.  
premix ( $\times 20$ )

92 1

48 1  $\times 25$  Ink to fig 7 (4°C) began 2:40 PM.

72 1

loaded 15  $\mu$ l each rxn into 0.8% TBE @ ~ 5:15 PM.

PCR screen of GFP100's

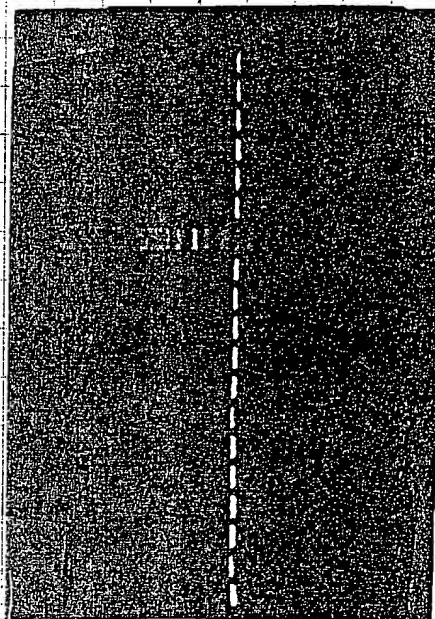
positive ID

GFP 100

GFP 100  $\Delta$ 

GFP 100 stop

all recombinant.

I streaked #1 and #2  
from each onto LB + Kan  
and inoculated 4 mL  
LB + Kan cultures, @ 37°C.

gfp 100 stop 1-6

gfp 100  $\Delta$  1-6

gfp 100 1-6

I inoculated 2 50 mL cultures (LBA): GFP 210  
GFP 210  $\Delta$ 

002295

I can visualize GFP via transient expression  
with these plasmids.

SIGNATURE

P. Kipp

NAME

P. Kipp

PURPOSE

50 mL plasmid preps  
miniprep GFP 100s

NOTES

1-24-97 start

DATE

1-29-97

50 mL plasmid preps - GFP 210, GFP 210 Δ

Span cells in 30 mL Corex tubes

- drained pellets

- followed protocol in blue book -

- 0.6 mL (each pellet) Sol'n I + lysozyme

- combine

- add 2.4 mL II → 10' ice mix by inversion

- add 1.8 mL III → 20' ice

- add 250  $\mu$ L  $\text{CHCl}_3$ , spin 10,000  $\times$ g 10' @ 4°C.

- collect super, add 0.6 vol isoprep - 15' RT

- spin 12,000  $\times$ g 15' at RT.- Rnase - added 2.5  $\mu$ L (10 mg/mL) Rnase to each prep

- @ 37°C ~ 2 hrs.

@ 2:30 I began the promase digestion according to the protocol.

GFP 100 minipreps -began during maxiprep lysis. I chose GFP 100 #1, Δ #2, stop #3,  
these cultures were more dense.

2.5 mL cells each. Sol'n I + Rnase

after recovering supernatant, I quickly  $\phi$ -OH/ $\text{CHCl}_3$  xt  
then  $\text{CHCl}_3$  xt.- these plasmids will be used to +F Appo, so I want them  
pretty clean.

002296

SIGNATURE

P. Kipp

PURPOSE

Conversation w/ Naomi - GFP  
-reorder 3' oligo

NOTES

1-29-97 (cont p 49)

DATE

1-29-97

Conversation w/ Naomi:

Two bases changes were identified in the 3' end of the GFP sequence; each make an AA change. These changes are present in all the clones previously ID'd as correct.

10	20	30	40	50	60
GGATCCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTCGAG 60					
CTGGACGGCGACGTGAACGGCCACAAGTTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCC 120					
ACCTACGGCAAGCTGACCCTGAAGTTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGG 180					
CCCACCTCGTGACCACCTTCACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCAC 240					
ATGAAGCAGCAGCACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACC 300					
310	320	330	340	350	360
ATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGAC 360					
ACCTTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTG 420					
GGGCACAAGCTGGAGTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAG 480					
AAGAACGGCATCAAGGTGAAGTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAG 540					
CTCGCCGACCACTACCAGCAGAACACCCCATCGGCGACGGCCCCGTGCTGCTGCCCGAC 600					
610	620	630	640	650	660
AACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCAC 660					
ATGGTCTGCTGGAGTTTCGTGACCGCCGCCGGGATCACTCACGGCATGGACGAGCTGTAC 720					
AAGTAAAGCGGCCGCCCGGGCTGCAG 746					

stop

The base in blue is an A in my sequences, which changes the AA at that position to Gln from Leu.

This sequence lies within the 3' primer that I designed. - see page 134 of IME #2. There is clearly a misplaced base in the primer sequence.

New primers - GFP 3' Sac 5' TTGAGCTCTTACTTGTACAGCTCGTCC 3'

5' ATCC ATG GTC AGC Ordered from GeneLink via email.

002297

I discarded the plasmids I was prep'ing.

SIGNATURE

P. Kipp

1-30-97 Start

1-30-97

I went through the  $-20^{\circ}\text{C}$  and discarded all of the plasmid stocks for GFP-gen, GFP-210, and GFP 100's.

I discarded all of my plates concerning the GFP cloning

I discarded all of the GFP bacterial stocks @  $-80^{\circ}\text{C}$ .

Made slides using Photostep -

1- fluorescent particles (green)

2- fluorescent onion cells (green)

3- fluorescent NT-1 cells (red)

4- sequence anomaly slide

I tried 2 different earth-tone backgrounds.

Liz was also making slides. They were imaged onto her film.

002298

SIGNATURE

P. Kipp

NAME P. Kipp

EXP. NO. ASSAY

PURPOSE

GFP Cloning

NOTES

1-31-97 begin

DATE

1-31-97

New GFP 3' Sac oligo arrived - 283 nmol  $\rightarrow$  resusp 280  $\mu$ l dH<sub>2</sub>OMutagenic PCR - as before (IME #2 pg 139)enough premix for 6 50  $\mu$ l rxns.

I made a 1:1000 dilution of GFPKS

I used 3  $\mu$ l of the diluted plasmid DNA

10X	5	40	Two rxns for each clone.	
MgCl <sub>2</sub>	3	24		
dNTPs	8	64	92°C	1
oligo 3'	0.5	4	48°C	1 x 25
tag	0.5	4	72°C	1
dH <sub>2</sub> O	29.5	236		
	46.5 $\mu$ l			

The PCR began @ 11 AM

I poured a 0.8% TAE gel w/ large wells to gel purify the PCR products.  
 - when the PCR ended, I loaded the gel

GFP,  $\Delta$ , SI shipping lanes.I ran the gel @ 78V for 45 min  $\rightarrow$  no photo

I isolated the bands, all were of strong intensity.  
 - gene-cleaned, eluted in 20  $\mu$ l.

I digested 10  $\mu$ l of the gel-purified product w/ Sac I in J  
 - 1  $\mu$ l enz. 20  $\mu$ l total  
 - @ 37°C ~ 3:30 PM.

002299

SIGNATURE

P. Kipp

PURPOSE

GFP Cloning  
ALS Seq. Results.

NOTES

1-31-97 (cont p 52)

DATE

1-31-97

GFP Cloning - After ~75 min of digestion time, I moved the digests to 65°C for 15'. This inactivates the enzyme, obviating the need to p-H.

- I brought the volume up to 100  $\mu$ L w/ dH<sub>2</sub>O
- added 34  $\mu$ L 3M NaOAc pH 5.2 + 300  $\mu$ L 100% EtOH.
- vtx, placed @ -60°C.

after ~20', I spin @ 14,000  $\times g$  for 12' at RT.

- washed pellet 500  $\mu$ L 70% EtOH.
- spin again 5' @ RT
- I was very careful in removing the supernatants

speed vac'd 2'

Resusp pellets in 17  $\mu$ L dH<sub>2</sub>O

- +2  $\mu$ L buffer D
- +1  $\mu$ L NcoI enz. → 37°C ~6:30 PM.

Naomi - Results of ALS Sequencing -

62	A1	(1-1)	CCA → ACA	Altered base seems to be shifted 5' of targeted nucleotide
	A2	(2-3)	CCA → TCA	
58	B1	(1-1)	CCA → ACA	roughly 30% of peaks are mutant base
	B2	(1-2)	CCA → ACA	
	B3	(2-3)	CCA → TCA	

All changes are to the intended base, just not at the intended base

Is there a problem w/ the ALS sequence or perhaps the sequence of the chimeras? Odd that chimeras 1 & 2 both show 5' change.

002300

The ALS-P (wild type) sequencing failed.

SIGNATURE

P. Kipp



NAME

P. Kipp

PURPOSE

ALS Seq.

GFP Cloning

NOTES

1-31-97 (cont' p 53)

DATE

1-31-97

ALS Seq (cont) - Naomi suggested that I send a fresh sample of each putative, and the wt to be sequenced again to confirm these results. She also wondered if I had another primer to use for sequencing since the ALS-1 primer binds a mismatch between the two alleles, it might lead to sequencing ghost bands.

I have the ALS 2 primer, but it failed in the PCR runs that I had tried earlier.

ALS PCR - I set up 6 100  $\mu$ l rxns.

genomics:	NI-1	}	primers 1 3 3
	1-1		
	1-2		
	2-3		
plasmid	1-3A#3	}	primers 2 3 3
	1-3A#3		

Used 5  $\mu$ l 1-3A#3 plasmid  $\rightarrow$  vol = 25

used 25  $\mu$ l each genomic sample, after heating @ 65°C, 5'

Made premix x 8 w/ primer 3 only

- added primer 1 or 2 separately after aliquoting premix

(25 DNA)			2nd
10x	10	80	added 1 $\mu$ l primer to each
Mg	6	48	
dNTPs	16	128	92°C 1
prim 3	1	8	63°C 1 x 25
Taq	1	8	72°C 1
dH <sub>2</sub> O	40	320	

link to 4°C soak file 002301

SIGNATURE

P. Kipp

NAME: P. Kipp

PURPOSE

GFP Cloning / ALS 2-3 + pks Cloning

NOTES

1-31-97 (cont p 54)

DATE

1-31-97

GFP Cloning - At 7:50 PM I moved the *Nco*I digests to 65°C to denature the enzyme - 15'

- brought volume to 100, added 33  $\mu$ l 3M NaOAc, 300  $\mu$ l 100% EtOH
- I also heated and reprecipitated the ALS 2-3 *Bam*/*Kpn* digested PCR fragment, vol to 100...
- ppt @ -80°C, roughly 30'
- spin 14,000  $\times$ g 15' @ RT
- carefully removed supernatant
  - washed w/ 500  $\mu$ l 70% EtOH, spin 5'
  - remove supernatant, pulse spin to re-collect
  - speed vac'd 2'

I resuspended each of the pellets in 10  $\mu$ l dH<sub>2</sub>O.

Ligations:  
 gfp-gen - 2  $\mu$ l ins, 1  $\mu$ l vect  
 gfp  $\Delta$  gen - " " "  $\rightarrow$  vol 10, -0.5  $\times$  C ligase  
 gfp stop gen - " " "

@ RT ~10:15 PM. The pgem52f *Nco*/*Sac*I vector was leftover from the last attempt to clone these products.

I also set up a ligation for the ALS2-3 PCR product.

4  $\mu$ l insert + 1  $\mu$ l pks *Kpn*/*Bam*  $\rightarrow$  10

@ RT.

002302

SIGNATURE

Peter Kipp



NAME P. Kipp  
PURPOSE GFP Cloning

NOTES

2-1-97 begin/end  
2-2-97 begin

DATE

2-1-97 / 2-2-97

GFP Cloning - I added 2  $\mu$ L of each of the GFP ligations and the ALS ligation into DH5 $\alpha$  via Eip'n.  $tc = 4.6$  for each.

1 hr. recovery @ 37°C, in YEN-B, began 3 PM.

I prepared X-gal plates 20' prior to plating by adding 50 mL 20 mg/mL X-gal to each plate.

pelleted cells, resusp in ~125  $\mu$ L YEN-B- added ~20  $\mu$ L 0.1M IPTG to each while resusp, prior to plating.

- plates @ 37°C o/n.

Kipp

2-2-97

GFP Cloning - at 8 AM, I inoculated 7 cultures, 3 of each GFP - from white colonies resusp in 20  $\mu$ L  $\rightarrow$  10  $\mu$ L inoc into 4 mL. There were no colonies for the ALS cloning.PCR screen - I screened 12 (4 ea.) colonies from the GFP clonings.

6 $\mu$ L cells -	2.5	10 $\times$		37.5	
	1.5	MgCl <sub>2</sub>		22.5	
	4	dNTPs	$\times$ 15 $\rightarrow$	60	92 1
	0.25	P2		3.75	46 1 $\times$ 25
	0.25	P1		3.75	72 1
	0.25	Taq		3.75	link 9°C
	10.25	dH <sub>2</sub> O		153.75	soak

PCR began @ 9:15 AM.

I returned at ~10 PM to plasmid prep the cultures and run the gel of the PCR screen -

plasmid preped using the kit, 1.5 mL cells each

002303

SIGNATURE

P. Kipp

P. Kipp

EXP. NO. ASSAY

57

PURPOSE GFP Cloning

NOTES

2-2-97 (cont p 56)

DATE

2-2-97

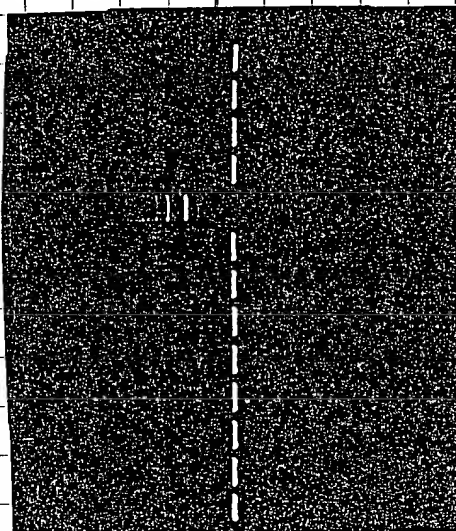
GFP Cloning - I loaded the gel (0.8% TBE) with the 12 PCR samples (~15  $\mu$ l) each. it ran while I finished the plasmid preps.

PCR screen of  
GFP-gems -

10'd + 5 for

gfp ✓

Stop ✓



} stop 1-4

}  $\Delta$  1-4

} gfp 1-4

All recombinant,  
as expected.

Eluted plasmids in 45  $\mu$ l dH<sub>2</sub>O, warmed to 60°C.

GFPs in pgen : 2  $\mu$ l in 100  $\rightarrow$  actual [ ] = 2.5  $\times$  260.

2-2

SAMPLE	A280	A260	280/260	260/280
1.0000	0.1002	0.1863	0.5379	1.8591 1
2.0000	0.0652	0.1150	0.5664	1.7655 2
3.0000	0.1689	0.3118	0.5417	1.8460 3
4.0000	0.0936	0.1702	0.5503	1.8172 $\Delta$ 1
5.0000	0.1187	0.2154	0.5510	1.8150 $\Delta$ 2
6.0000	0.0939	0.1694	0.5543	1.8041 $\Delta$ 3
7.0000	0.0719	0.1257	0.5722	1.7475 S1
8.0000	0.1382	0.2484	0.5561	1.7982 S2
9.0000	0.1522	0.2782	0.5472	1.8274 S3

Yields variable, but sufficient

Stored samples @ 4°C O/N.

002304

SIGNATURE

Peter Kipp

NAME

P. Kipp

NOTES

PURPOSE

Nuclear XT  
GFP Cloning

2-4-97 (cont p 58)

DATE

2-4-97

Repeated grinding for UV treated sample -

added an additional 30-40  $\mu$ L NIB to each

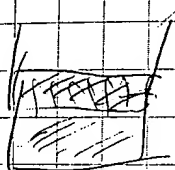
The mixtures look very chunky.

Polytron - 3 x 30 sec bursts

after polytroning 2x, added ~50  $\mu$ L buffer  
mixtures turned to applesauce from chunky  
after 3rd polytron, back on ice

each sample done separately, polytron cleaned between

While on ice, the NT-1 sample formed a layer of ice  
that was thicker and whiter than the greyish bottom layer



The mixtures were filtered through 2 layers  
of cheesecloth into fresh beakers

The flow throughs are greyish green. A portion of the  
material did not go through. It was white and thick.  
After cheesecloth, there were no clumps

Filtered through single layer of <sup>micro</sup> cheesecloth; again some material  
left behind, dramatically less.

Spin in 250  $\mu$ L bottles - GSA @ 4300 rpm (3,000  $\times$  g), 10' @ 4°C.

- decanted supernat. pellets tricolored black, brownish-green, grey

- resusp each pellet in 150  $\mu$ L NWB

- scraped pellets off sides w/ 10  $\mu$ L pipet, mixed by gentle  
stirring and slow pipetting.

- spin @ 4300 rpm in GSA, 5' @ 4°C

002305

SIGNATURE

P. Kipp

NAME P. Kipp

EXP. NO. ASSAY

PURPOSE Nuclear XTs

NOTES

(2-3-97)

2-4-97 (cont p59)

DATE

2-4-97

(XT's cont) - Resusp. washed pellets in 50 mL lysis buffer by swirling pellets w/ pipet, gentle pipeting.

- moved each sample to 2 x 50 mL Falcon tubes

- add  $\frac{1}{10}$  (2.5 mL) 4M  $\text{NH}_4\text{SO}_4$

- placed tubes horizontal in ice on shaking platform @ 75 rpm

- began @ 3 PM, time varies from 30' to 1 hr.

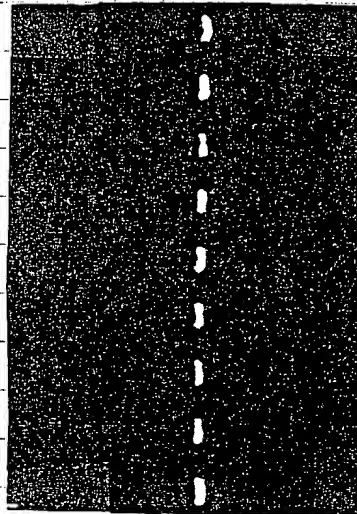
GFP Cloning - This AM prior to starting the extraction, I set up nine GFP ligations with the fragments that I purified yesterday.

From 2-3: I digested <sup>20 ng</sup> each gfp-gen plasmid w/  $\text{Sma I}$ , followed after ppt and resuspension, with  $\text{Nco I}$ .

From 2-3

Isol. of GFP ~~20~~ gen  
N/S frags for cloning  
into 210.1

purified by gene-clean  
eluted in 20  $\mu\text{L}$   $\text{dH}_2\text{O}$ .



} gfp stop 1-3

} gfp  $\Delta$ 1-3

} gfp 1-3

Yesterday, I also performed an A/S genomic PCR reaction to send frags to Naomi for sequencing

PCR: 25  $\mu\text{L}$  aliquots of genomic DNA, 5  $\mu\text{L}$  samples of ACS1-3P (1:1000)

I set up 6 rxns:

NT-1

1-3P

1-1

1-2

2-3

1-3P

} primers 1, 3

- primers 2, 3

002306

SIGNATURE

P. Kipp

NAME P. Kipp

PURPOSE

GFP/ALS cloning  
Nuclear Extracts

NOTES

(2-3-97 cont)  
(2-4-97 cont p60)

DATE

2-4-97

2-3 (cont) -

92 1

63 1 x 25

72 1

The PCR was from a premix x7 - 100  $\mu$ L reactions.Ran 40  $\mu$ L each reaction on a TBE gelALS PCRs -

2-3

1501. frags for NT

1-2

1-1

1-2

1-1

1-3P

2-3

confirm that primer 2  
works

ALS 1-3P, 2-3

ALS 1-3P, 1-3

1 isol. these fragments  
and ran them on a TBE  
gel compared to the Kb ladder

NT-1

Each fragment was eluted in 20  $\mu$ L dH<sub>2</sub>OGel not shown - Estimated concs = NT - 5 ng/ $\mu$ L1-1 7.5 ng/ $\mu$ L

1 sent 20 ng each product

1-2 6 ng/ $\mu$ Land ~1.1  $\mu$ g each GFP plasmid2-3 4 ng/ $\mu$ L

(S3=9... gfp1=9)

1-3P 20 ng/ $\mu$ L

End 2-3

2-4 GFP Cloning - The ligations to form GFP 210s each had  
1  $\mu$ L of insert and 0.75  $\mu$ L 210.1 vector, 1  $\mu$ L 10X, 0.7  $\mu$ L ligase  
total volumes 10  $\mu$ L, @ RT. They began @ ~9 AM 002307

SIGNATURE

Peter Kipp



NAME

P. Kipp

PURPOSE

Nuclear extracts  
GFP Cloning

NOTES

2-4-97 (cont p 61)

DATE

2-4-97

Nuclear XTS - After ~ 50' shaking @ 0°C, the samples were very goopy from the lomal DNA release. They were still clear, just very viscous.

Ultra centrifugation - 1 hr @ 25,000 in SW28

We brought the volumes up to 38 ml each so that the plastic tubes wouldn't collapse during centrifuge.

This add'n of NAB - about 10 ml each 25 ml aliquot, decreases the overall  $[NH_4 SO_4]$  which we will have to take into account during the  $NH_4 SO_4$  cut after spinning.

GFP Cloning - Epp'n 2  $\mu$ l each ligation into D115a.  $t_0 = 4.5-4.6$   
plated LBA after 60 min recovery. except D2 which arched.

placed plates @ 37°C O/N.

Nuclear XTS - After ultra spin, we measured the supernatants and moved them to 125 ml flasks. Both are perfectly clear.

NT - 71 ml  $\rightarrow$  34.36 g  $NH_4 SO_4$   
UV - 70 ml  $\rightarrow$  33.80 g "

to reach ~85% saturation (at 0°C), we will add  $NH_4 SO_4$  (above) solid slowly with constant stirring. We assumed 5% saturation at the start (actually it was ~7%).

Added salt very slowly over ~50' @ 0°C.

- checked pH periodically, no change throughout, constant btwn 7-7.5

- after all in, stirred add'l 30' on ice.

002308

SIGNATURE

P. Kipp

NAME P. Kipp

PURPOSE

Nuclear XTS.

NOTES

2-4-97 (cont' pg 62)

DATE

2-4-97

XTS (cont) - ppts - clear supers, quite cloudyspin ppts @ 10,000 rpm in SS-31 rotor (12,000 x g) 10' @ 4°C  
in 2 x 30 mL baked corex tubes.

- decanted supernats. resusp pellets in 2 mL NEB
- wouldn't go into solution, added add'l 2 mL NEB
- very viscous, but in solution

Inserted 2 mL into each of 2 slide-a-lyses (10,000 MW co)  
for both samples 4 slides total

Dialyzed against ~2L NEB at 4°C. Began 7:10 PM.

NEB: 25 mM Hepes/KOH pH 7.6

100 mM KCl

0.1 mM EDTA

5 mM  $\beta$ -mercapto

1 mM PMSF

10% v/v glycerol

Made 2L - @ 0.5 mM PMSF

Dialyzed until 9:30 PM. GM spin @

- we froze the supernatants in 100  $\mu$ L aliquots in dry ice / EtOH bath and stored them @ -30°C

- I marked the last aliquot of each to indicate that it was less than 100  $\mu$ L.

NEW ALS primer: ALS-4: 5' TTGAATTCAGCGGCCTCGCTGACGG 3'

EcoRI

same as ALS-2, w/ RI site

Also ordered 35S 5': 5' GAA GTGACAGATAGCTGGGC 3'

002309

SIGNATURE

Peter Kipp

NAME P. Kipp	EXP. NO.	ASSAY
PURPOSE GFP Cloning Seq. results	NOTES 2-5-97 start	
		DATE 2-5-97

GFP Cloning -  $\Delta 2$  has no colonies, all others have > 75

I selected 3 colonies from each plate to see if they have the insert - in anticipation of the sequencing results.

1 gfp-KS treated Kera tag (0.2, 0.5, 1  $\mu$ L) on gfp-KS.  
24 210 putatives

Set up premix for 30 rxns. using 5' Nco and 3' SAC.

75 $\mu$ L 10x	mixed, aliquoted Kera 3 rxns,
45 $\mu$ L $MgCl_2$	then added 6.75 $\mu$ L tag and
120 $\mu$ L dNTPs	aliquoted mine
7.5 $\mu$ L 5' Nco	1' 92 C.
7.5 $\mu$ L 3' SAC	1' 48 C. x 25 cycles. began 1 PM
308 $\mu$ L $dH_2O$	1' 72 C.

Naomi - ALS sequencing results are confirmed: 1-2  $\rightarrow$  ACA

1-1  $\rightarrow$  ACA

The ALS 2 primer failed in the sequencing. 2-3  $\rightarrow$  TCA

She hasn't had time to fully analyze the GFP sequences.

I asked her to email me the raw data, I can do it.

The sequencing did not go well. The T7 worked on two plasmids, and the SP6 worked on 6:

T7: # 1 (S3)  
5 ( $\Delta 2$ )

SP6: 2 (S2) 7 (gfp1)  
3 (S1) 8 (2)  
5 ( $\Delta 2$ )  
6 (di)

no sequence on 4 or 9.

002310

SIGNATURE

P. Kipp



NAME

P. Mapp

NOTES

PURPOSE

GFP Cloning  
GFP/ALS Sequencing

2-5-97 (cont p64)

DATE

2-5-97

I loaded a TBE gel w/ 15  $\mu$ l of the PCR reactions -

top: gfp, K1 K2 K3, gfp1-1 1-2 1-3 2-1 2-2 2-3 3-1 3-2 3-3

bottom: gfp, A1-1 1-2 1-3 3-1 3-2 3-3, S1-1 1-2 1-3 2-1 2-2 2-3 3-1 3-2 3-3

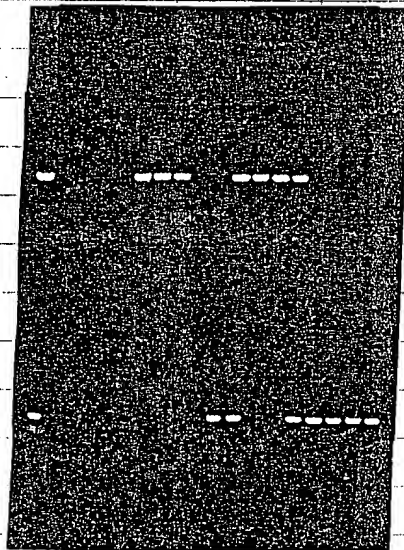
While the gel ran, I put the GFP-gen SP6 sequences into EditSeq  
and R3C to facilitate aligning them. I printed out all the  
sequences to take them home to study.

PCR screen of GFP-210  
putatives:

ID gfp

Stop

→ no deltas



FedEx to Kimeragen -

ALS-1, ALS-3 @ 100  $\mu$ l,  
H1, 1-2, 2-3 frags. Patricia  
will clone them after reamp  
into T-tailed vector.

GFP sequencing analysis - #1 (Stop3) - no reiterated A

#2 (Stop2) - missense

#3 (stop1) - missense

#5 (B2) - missense

#6 (B1) - good, but incomplete @ 5'

#7 (g1) - missense

#8 (g2) - good, but incomplete @ 5' &amp; 3'

002311

SIGNATURE

Peter Mapp

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